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SINGLE CELL PROTEINS FROM CELLULOSIC WASTES

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The need for protein feed and the need to dispose of waste cellulose are problems being simultaneously studied at Louisiana State University where research has been conducted to develop a feasible method for the direct conversion of cellulosic wastes to microbial protein---a single cell protein to be used as an animal feed supplement. This report presents data on the process and summarizes their research in large-scale production. The economics and techniques of the conversion process are compared with work done by petroleum companies on the fermentation of hydrocarbons. Controlling variables utilized in the process are defined and significant goals necessary to achieve success in the field are described. The research deals mainly with the alkali treatment and fermentation parts of the process. Different micro-organisms from which single cell protein can be produced are given. Research in the areas of cell flocculation, flotation, or hyperfiltration are recommended to improve the most costly single step of the process--drying the cells. Methods of decreasing the degree of crystallinity are also included. The practicality of complete acid hydrolysis of cellulose to a soluble substrate for conversion to yeast protein and partial acid hydrolysis of the cellulose followed by microbial consumption is discussed. An economic analysis is given of the potential markets and the competitive cost with other proteins. Producing techniques for feed rather than for food as a means to reduce costs is suggested.

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FROM CELLULOSIC WASTES

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment - air, water and land. The multidisciplinary programs of the National Environmental Research Centers provide this focus as they engage in studies of the effects of environmental contaminants on man and the biosphere and in a search for ways to prevent contamination and recycle valuable resources.

In an attempt to solve the problem of solid waste disposal, the study described here employed a large-scale production method of converting waste cellulose directly to protein through the use of cellulolytic bacteria. At the same time the study helped to alleviate the problem of protein deficiency since the new protein product could be used as a source of animal feed.

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Director, National Environmental
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PREFACE

Cellulose is continuously being replaced by the process of photosynthesis and therefore represents the world's only inexhaustible raw material. Because it also represents a relatively cheap starting material for an infinite number of products, its use is reaching tremendous proportions. Like other cheap starting materials, it is usually easier to get virgin compounds than to recycle part of that which has once been used. This causes a very real waste disposal problem.

Multiple stomached animals have been consuming cellulose for millions of years and converting it to protein. Because this basic process is so slow and inefficient, and cannot keep pace with the population explosion the world now faces a gigantic protein deficiency. This paradox of too much feed and too little protein is the subject of this research report.

Louisanna State University working under both a contract (NO. PH86-68-152) and a research grant (EC-00328) is attempting to solve these two problems by converting waste cellulose directly to protein through the use of cellulolytic bacteria. This report presents data to show that such a conversion process is many orders of magnitude faster and more efficient than protein production from ruminants.

The grant has been supervised by Mr. Louis W. Lefke and Mr. Daniel J. Keller through the Federal solid waste management program. The contract was supervised by Dr. Thomas C. Purcell and Mr. Clarence Clemons of the same office.

Futher information about this process can be obtained from our report (SW-24C) on the construction of chemical-microbial pilot plant.

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Summary

There is a huge potential market throughout the world for single cell protein as an animal feed supplement. This market is a direct result of the protein difficiency caused by the recent population explosion. Several of the world's largest petrochemical companies such as Imperial Chemical of England, British Petroleum of England, Standard Oil of the U.S., Gulf Oil of the U.S., Chinese Petroleum of Taiwan and many others have recognized this marketing opportunity and have spent millions of dollars and thousands of man-hours of research effort to develop a product to fill this need. The current leader appears to be British Petroleum with the recent construction of a 6.5 million dollar plant at Lavera, France to produce 16,000 tons per year of yeast from hydrocarbons. The construction of this plant culminates a 16.5 million dollar expenditure in basic research on the fermentation of hydrocarbons.

The objective of the research reported here is to reach this same market with a single cell protein product produced from cellulosic waste. This approach utilizes a more desirable starting material because its consumption could offer a partial solution to the solid waste disposal problem and because the world is almost as short of fossil fuels as it is of protein. The basic problems involved in developing a process to use waste cellulose as substrate for fermentation may be greater than were the problems encountered in using hydrocarbon as a feed stock because of the tremendous analytical problems caused by the heterogeneous nature of the cellulosic waste. Our results to date appear promising although a much more modest expenditure than 16.5 million dollars has been invested.

To develop a fermentation process, one must be able to evaluate the

response to any change in a variable that has an effect on the process. Such a response might be cell concentration, soluble carbohydrate, residual substrate, etc. Yet native cellulose contains varying amounts of non-biodegradable lignin whose presence renders almost every standard analytical procedure completely invalid. This highly colored material changes its base color with any infinitesimally small change in processing conditions in such a manner as to render questionable many measurements.

Still a careful evaluation of a compilation of large quantities of data acquired by the project investigators shows not only that this product could possibly be produced competitively with yeasts from hydrocarbons but that the product may be more desirable in terms of total protein content and amino acid distribution.

The economic analysis that is reported here shows that the capital investment required to produce the same amount of product as British Petroleum's plant will be about 4.2 million dollars. The product cost which includes 6% interest on capital, 10% depreciation costs and 10% return on investment, will be about 10.0 cents per pound or a little over 18 cents for a pound of pure protein. This is a few cents a pound cheaper than the anticipated base cost of yeast. But yeast has only 52% protein compared to 55% or more protein for these cellulolytic bacteria. Yeast from hydrocarbons may have difficulty competing on the basis of cost per pound of pure protein.

This report will summarize our research in large scale cell production since our efforts on the isolation and culture of the microorganisms have been described in a previous report. We have designed and built a pilot-plant which has enabled us to scale-up the fermentation process by several orders of magnitude. The data obtained from this operation indicate the process will probably be economically feasible but many aspects of the

project require further extensive investigation. To date, we have defined the controlling variables in the process and set the goals necessary to achieve success. Each of these goals has been met independently during the course of this research but the task ahead requires an integration of these goals into an optimum complete process. This objective can be attained if further support of this work is forthcoming.

Introduction

The purpose of this work was to develop a feasible method for the direct conversion of cellulosic wastes to microbial proteins. Such a conversion process should help not only with the serious problem of solid waste pollution but should also help with the solution to the worldwide protein deficit.

The world appears to be more ready to accept direct microbial conversion as a means of producing more protein with or without the benefits to waste disposal problems. To demonstrate this fact, British Petroleum (BP) has just placed on stream a 7 million dollar plant at Lavera, near Marseilles, France (1). The facility has a deisgn production capacity of 16,000 tons per year of single cell protein (SCP). The plant uses gas-oil as a feed stock to grow yeast aerobically. These microorganisms consume the n-parraffins present in the feed stream and leave the branched hydrocarbons. The product from the plant is to be used as animal feed although no one is discarding the possibility of using it for human food.

Imperial Chemical Industries (ICI), England's largest chemical company has been so impressed with the success of BP that they have launched a similar development program to find a bacteria that will economically consume natural gas as well as petroleum. For a world increasingly short of fossil fuels, this would seem like a somewhat poor choice of starting materials for both of these companies. Particularly if waste cellulose could be used as the feedstock to produce single cell protein.

It is interesting to note that BP has already spent over 16.5 million dollars on the research phase of their development to produce yeast on hydrocarbon (2), while the process being reported here with its inherent ecological advantages has received considerably less attention. The problems

associated with the utilization of waste cellulose by microorganisms are more complex than are those associated with the utilization of hydrocarbon. How, for example, does one determine the biomass produced when this measurement has to be run in a highly colored base solution of lignin, ureacted cellulose, nutrients, microbial cells, etc. whose color changes continuously? In such a system, the usual monitors of cell mass; color, turbidity, protein analysis, etc. means very little because the solution contains a large amount of suspended cellulose which hinders most analytical procedures. When these suspended solids are removed, part of the product goes with them, which means problems in the subsequent analytical step.

When the process streams contain large amounts of solids, as they necessarily must to attain high cell concentrations, these solids cause severe pumping problems. At every point in the system where flow velocities are preceptibly decreased the lines plug which in turn cause upsets in the system.

It would appear then, that the development of a process to produce single cell protein on hydrocarbons may be somewhat easier than the development of a process to consume waste cellulose. However, the hydrocarbon people are certainly not without problems. Yeast in both food and animal feed is not new, but only in the last 10 years has their potential caused an extraordinary amount of interest (3). The yeast that was formerly used in diets was probably produced on carbohydrates because that produced on petroleum may contain benzypyrene, a carcinogenic impurity which generally must be limited to less than one part per million in the feed stream to the fermenter (4). The protein product must also be carefully washed to remove any aromatics that may be present.

In Japan, the AJINOMOTO Co. produces nearly 100,000 metric tons a year of yeast grown on hydrocarbons (5). This product is generally used as animal feed because the high cost of purifying the raw material to a point where it will pass the benzpyrene requirement for human food is prohibitive. Obviously the benzpyrene limitation is less rigorous for feed grade petroprotein.

If an agricultural waste cellulose such as bagasse is used as the feed stream to grow microorganisms, there is very little possibilty that this material would contain any toxic substances since it is a link in the natural food cycle for both man and animals.

Bacteria vs Other Organisms

Single cell protein can be produced from a number of different microorganisms. Green algae (eucaryotic) and blue-green algae (procaryotic) or blue-green algae, Spirulina maxima are all possible candidates. The Spirulina maxima has been used as essentially the only protein source for many years by peoples in the Chad Republic in Africa. Still, algae have rather poor protein composition and are not very readily digestible.

Fungi has also been studied as a possible source of single cell protein. These organisms show particular promise because of their highly active enzyme systems. They can degrade native cellulose. They also generate large amounts of mycelium which makes fungi easy to harvest. The difficulties with fungi are the long doubling times of several days to 2 weeks, the rather poor digestibility of the protein, and the low total protein content. Several different fungi are truly cellulolytic, that is they generate both the C_1 and C_x enzymes postulated by Reese and co-workers be necessary for the complete breakdown of cellulose to simple sugars (6).

Yeast cells usually have associated with them various necessary vitamins and have long been used as a flavoring agent. They are easy to recover from the fermentation media, have reasonable doubling times of 2 to 6 hours, and are extremely large cells when compared to bacteria. Perhaps the greatest trouble with yeast is the thickness of their cell walls which makes them quite resistant to digestion by single stomached animals. No yeasts are currently known that possess the entire system of cellulase enzymes necessary for the utilization of waste cellulose. The single cell protein being produced in this study is a bacteria that was originally isolated by V. R. Srinivasan and Y. W. Han (7) from a sugar cane field in the Baton Rouge area. Bacteria are thought by many people, including ICI as mentioned earlier, to be a better source of SCP than any other organism. The following excerpt is taken directly from PAG Statement No. 4, Single Cell Protein, by the Protein Advisory Group to FAO/WHO/UNICEF.

> Bacteria would possess a number of advantages over other organisms. They have higher growth rates and much higher protein contents. They have disadvantages of being susceptible to phage, having higher nucleic acid content and being less well known in human nutritional experience. However, they are a good long range prospect because of the potential economic advantage they offer.

Materials and Methods

In order to investigate the possibility of growing bacteria on the cellulosic portion of urban solid waste, a pilot plant was constructed which uses bagasse as feed. It was reasoned that bagasse, the residue from the sugarcane stalk after the sugar has been extracted, would represent a substrate that might be similar to the cellulose found in urban solid waste. Because of the large amount of lignin and other non-cellulosics in the stalk it may be somewhat less desirable to microorganisms than say, newspaper which contains some partially purified wood pulp. The proximate analysis of various agricultural cellulosic materials is given in Table 1. These data show that for bagasse, for example, only a little over one half is actually cellulose. This means that if the microorganisms are so selective that they consume only the anhydroglucose residues then they would be expected to consume not more than this amount. If the typical conversion ratio of 50% yield of microbial cells from carbohydrate is adhered to, this means that one might expect to obtain 25 lbs. of cells per 100 pounds of bagasse fed. This latter figure is based on sufficient residence time in the fermenter to obtain complete conversion, which also is not a very desirable situation since it would lead to unduly large fermenters. Actual material balances around the fermenters show that this reasoning is not quite correct and that about 0.38 pounds of cells are produced for each pound of substrate solubilized instead of 0.25. The interpretation is that the microorganisms are in fact consuming some components that are not necessarily beta 1, 4 anhydroglucose units tied into the insoluble cellulose chains.

Any new plant may be designed on the basis of consuming a certain amount of raw material per unit of time or on the basis of producing a given amount of

TABLE I. PROXIMATE ANALYSIS OF AGRICULTURAL CELLULOSICS

Substrate	Protein	Fat	N.F.E.*	Fiber	Lignin	Ash
Cotton linters - raw	2.45	0.96	4.48	85.7	5.0	1.27
Cotton linters - purified	3.27	1.69	3.69	90.0	0.84	0.42
Bagasse - whole	2.68	1.0	31.1	52.74	10.63	1.79
Bagasse - pith	2.97	5.23	30.83	47.14	9.28	4.52
Bagasse - fiber	1.74	0.76	32.78	52.39	10.23	2.06
Rice straw	5.05	2.41	36.70	34.39	4.06	17.36
Sawdust - pine	0.42	2.89	13.16	74.83	8.45	0.21
Johnson grass	6.71	2.87	39.44	37.00	5.01	8.95
Prairie grass	5.32	2.06	41.84	36.73	7.39	6.63
Alfalfa meal	22.11	2.38	39.46	23.88	5.39	6.74
Cottonseed hulls	5.53	1.51	15.83	50.54	23.31	3.25
Corn cobs	2.61	0.87	46.89	37.10	10.44	2.06
Oat straw	7.74	2.0	35.50	36.94	7.41	10.39
Wheat straw	2.71	1.25	35.58	46.08	8.02	6.36
Sorghum bagasse	2.73	2.73	41.60	40.54	7.17	4.21

*N.F.E. is Nitrogen Free Extract

product per unit of time. According to the above discussion then at a retention time sufficient to obtain about 80% solubilization of the initial feed, about 0.28 lbs. of product will be produced per pound of feed or roughly 3.5 pounds of bagasse feed to obtain a pound of cells. Attention should be called to the fact that a better quality of feed such as writing paper, would give a much larger yield per pound of input since more of this material is cellulose. In any event, the yields quoted for bagasse represent an 80% reduction in volume.

If bagasse is to be used as feed to the process then it would have to be charged out at its fuel replacement value of 0.25 cents per pound. This would mean that about 0.875 cents would have to be charged for each pound of cells produced. On the other hand, disposal costs for urban solid waste after delivery to a site are rarely less than $3.50 per ton and may go as high as $10.00 per ton. Typical urban solid waste contains 50-60% cellulose, and if the proportionate amount of this is credited to the fermentation process a negative cost of 0.1 to 0.3 cents per pound of cellulose will be realized.

Alkali Pretreatment

The bagasse must be pretreated in some manner to obtain a high degree of digestion by the microorganisms used in this study. This is attributed to the fact that cellulomonas does not possess a swelling enzyme. Reese, et al (6) have shown that many fungi are truly cellulolytic and generate the C_1 and C_x enzymes necessary for the complete hydrolysis of cellulose. The C_1 enzyme might be considered a swelling enzyme which would be necessary to cause disruption of the crystal lattice of native cellulose. Their function appears to be one of wedging apart the cellulose molecules to break the strong Van der Waal forces that normally hold the molecules in a fixed spatial arrangement.

Without this action, the hydrolyzing C_x enzymes have difficulty in making effective contact with the cellulose molecules. In any case, a chemical pretreatment that causes decrystallization will greatly improve the rate of enzymatic hydrolysis by increasing the number of active sites available for attack.

Native cellulose is also accompanied by various amounts of lignin, a highly colored polymer that is extremely resistant to microbial decomposition. One of the purposes intended by nature for this material is to serve as a binding agent to enclose the cellulose molecules into fibrils. Molecules surrounded by such casings would be extremely resistant to contact by invading enzymes or organisms. Cowling (8) has suggested that invading microorganisms must not only be able to hydrolyze cellulose but must also be able to depolymerize lignin if the rate of attack is to be substantial. Lignin, when depolymerized, is soluble in alkali but essentially insoluble in acid solutions. Another reason for chemical pretreatment, then would be for depolymerization and solubilization of the lignin in native cellulose. From these remarks one might conclude that acid pretreatment may be less effective than alkaline pretreatment of the cellulose if microbial utilization is the intended end use.

Cowling has shown in his excellent review of the causes for the susceptibility of cellulose to microbial attack that if cellulose is partially hydrolyzed by acid, the hydrolysis takes place in the amorphous region thereby causing an increase in the degree of crystallinity of the remaining insoluble cellulose. This results in a large decrease in the rate of microbial consumption of the insoluble portion. Data listed in Table IA are the results of experiments conducted in our own laboratory to compare the effect of acid and alkali treatment on microbial digestion and clearly confirm Cowling's conclusions.

The cellulosic waste then should probably be chemically pretreated with

Table IA

Effect of Acid and Alkali Treatment on the Digestibility of Bagasse

	Treatment [a,b]		Liquid		Solid		
	1st	2nd	Total Sugar (mg/g)	Reducing Sugar (mg/g)	Recovery %	Digestibility[c]	Lignin (%)
ACID	0% A, 15'	1%, 15'	34	16	80.5	-	9.44
	10% A, 15'	1%, 15'	91	16	55.6	-	20.52
	50% A, 15'	0.5% A,15'	176	120	51.6	0.3	36.78
	50% A, 15'	1% A, 15'	232	168	53.4	0.3	33.52
	50% A, 15'	1% A, 1hr	230	140	50.0	0.2	46.88
	50% A, 15'	1% A, 2hr	212	192	53.0	0.2	38.64
	50% A, 15'	2% A, 1hr	146	86	49.9	0.3	39.78
	50% A, 2hr	10% 1.5hr	143	167	68.6	0.3	16.20
ALKALI	0% B, 10' (R.T.)	-	22	7	86.3	0.4	7.74
	0.5% B, 10' (R.T.)	-	22	8	83.2	0.55	10.18
	1% B, 10' (R.T.)	-	25	9	76.2	0.6	9.78
	5% B, 10' (R.T.)	-	32	13	72.5	0.55	9.76
	10% B, 10' (R.T.)	-	36	16	64.3	0.6	8.18
ACID&ALKALI	0% B, 15'	1% A, 15'	35	6	62.8	0.4	16.98
	1% B, 15'	1% A, 15'	98	6	77.1	0.3	16.56
	5% B, 15'	1% A, 15'	92	29	41.0	-	54.14

[a] A = H_2SO_4; B = NaOH; RT = Room Temperature [b] Heated in autoclave unless otherwise indicated.

[c] Digestibility = Degree of substrate solubilization after 3 days fermentation

an alkaline hydroxide. Table II shows that rapid assimulation of the cellulose by rumen microorganisms occurs as a result of this treatment. However, if the cellulose is presoaked in alkali and the excess is squeezed out, this would still leave a very strongly alkaline slurry which in general would be too high in OH-ion concentration for microbial growth. Therefore one is left with two choices. Either wash out the NaOH remaining after the treatment, or neutralize with an acid. To wash out the remaining caustic means that the alkali soluble carbohydrates would also be removed with the caustic, causing the loss of part of the substrate which would otherwise be available to the microorganisms. The remaining alternative of neutralization with an acid is probably the only acceptable answer. However, this also has disadvantages of either limiting the strength of the caustic used in the treatment or the concentration of the substrate in the feed stream. This comes about because the salt formed by neutralization must not become too high in concentration to support good microbial growth in the subsequent fermentation step.

The presence of excessive amounts of sodium chloride in the menstrum has been found in our experiments to be detrimental to microbial growth. The optimum amount is approximately 3.0 g. per liter while twice this much is deleterious even though 6.0g/l was originally used in the isolation media. Furthermore, the presence of salt in the menstrum can lead to high salt content in the product, depending, of course, on how the cells are harvested, and whether or not the cells are washed before drying. One of the real advantages of this approach to protein production compared to that using a hydrocarbon substrate is the lack of a required washing step on the harvested cells. It would, therefore, be detrimental from a cost standpoint to have excess salt.

A typical alkali treatment would use 3% caustic solution to treat a ground cellulosic substrate. The excess NaOH after treatment is expressed by passing

Table II

Corrected Average Cellulose Digestibility Values
With Rumen Fluid

Substrate	Cellulose Digestibility % dry cellulose digested Untreated	Treated
1. Cotton Linters - Raw	0.82	5.4
2. Cotton Linters - Pure	31.7	21.4
3. Bagasse - Whole	15.1	57.0
4. Bagasse - Pith	26.5	55.0
5. Bagasse - Fiber	30.1	50.3
6. Rice Straw	7.3	54.1
7. Sawdust - Pine	4.25	-
8. Johnson Grass	66.5	88.0
9. Prairie Grass	16.2	45.7
10. Alfalfa Meal	55.7	53.5
11. Cottonseed Hulls	9.8	35.5
12. Corn Cobs	19.3	44.0
13. Oat Straw	35.5	66.0
14. Wheat Straw	25.4	44.0
15. Sorgum Bagasse	30.2	61.5

NOTE: Alfalfa digestion control for all runs was corrected to 50.0% digestion. The digestibility values for the various cellulosics were corrected proportionally. Significant values of each set were averaged to obtain the values reported. Data taken from Proteins from Waste Cellulose by Chemical Microbial Processing, by C. E. Dunlap. A dissertation the Chemical Engineering Department at Louisiana State University.

the solid between rubber squeeze rolls or a mechanical pressing device such as a screw-press. The amount of pressure applied by the squeezing device will determine how much caustic will be left with the cellulose to be neutralized by acid. A typical composition after squeezing through 3-inch diameter rubber rolls would be 40% dry bagasse and 60% caustic solution. If the caustic is neutralized with hydrochloric acid, this would require 4.10 pounds of HCL and would produce 6.57 pounds of salt per 100 pounds of bagasse fed. Since approximately 300 pounds of bagasse must be fed to produce 100 pounds of cells; this means that about 20 pounds of salt will be produced for every 100 pounds of cells.

One way to prevent the production of so much salt with the corresponding consumption of hydrochloric acid is to squeeze out more caustic solution. This appears to be feasilbe by using an apple squeezer that has recently been installed at the pilot plant. This unit applies more pressure for longer periods of time and is able to get to about 55 pounds of caustic solution for every 45 pounds of dry bagasse produced. A typical French Oil Screw Press would undoubtably bring this down even further to about 42% NaOH solution which would be highly desirable because this would cut the final salt content to about 3.2 pounds of salt per 100 pounds of bagasse or a decrease of almost one half.

It is interesting to note that a French Oil Screw Press would also have some other remarkable advantages over our present system of preparing alkali cellulose. Since delignification and decrystallization are a function not only of caustic concentration but also of time and temperature this unit would be ideally suited to apply all 3 functions in one single step. That is, a screw press because of the friction involved in forcing the alkali cellulose along the barrel would cause heat build up. This heat release would cause a corresponding temperature rise. Since most such mills are equipped with

variable speed drives, the time at this elevated temperature could be set independently of the other 2 variables. Han (9,10) has shown that this step would also cause sterilization of the bagasse so that the product could be injected directly into the fermenter thereby eliminating the pumping and sterilization step.

Another way to greatly decrease the required alkali treatment is to use prepurified waste paper such as writing paper, books, packaging paper or other types of paper that one might normally find in urban solid waste. This type of paper is partially digestible by cellulomonas without an alkali pre-treatment since it has already had a chemical digestion step. This is not quite true for newspapers because they normally contain 75% ground wood pulp. Experiments in both the pilot plant and the laboratory using untreated computer printout paper have been reasonably successful. A comprehensive report on the composition of the cellulose protion of urban solid waste is contained in Golueke's report to the Bureau of Solid Waste Management (11)

Fermentation

The fermentation step being carried out here is aerobic and the carbohydrates are converted to cell mass with approximately 50% yield. The composition of bagasse has been found by the Feed and Fertilizer Control Laboratory of Louisiana State University to be:

2.68% protein
1.00% fat
31.10% nitrogen free extract
52.74% fiber (cellulose)
10.63% lignin
1.79% ash

Thirty eight pounds of cells are produced for every 100 pounds of solid consumed. This means that approximately 20 pounds of the nitrogen free extract is being consumed by the microorganisms and all of the protein and fat. This also says in effect that the nonbiodegradable discharge from the plant will run about 24% of the total solid feed. About one half of this discharge will be lignin.

Tentative and rather incomplete experiments have shown that it may be possible to recover a large portion of the lignin by adjusting the pH of the effluent from the fermenters downward. This causes insolubilization and floating of the lignin which can then be removed by skimmers.

Aerobic fermentation requires that large amounts of oxygen be diffused from the air to the liquid media where the microorganisms are growing. The amount of oxygen required by the organisms to oxidize a particular substrate will largely determine the power requirements of the fermenter. In the case of carbohydrates, this amount is only about one third that required to oxidize n-paraffins because the cellulose is already partially oxygenated. This means that not only are the power requirements for agitation considerably less but so is the total amount of air that must be pumped to the carbohydrate fermenters.

The actual design of the fermenter has a great deal to do with the amount of oxygen transferred per pound of air introduced (12). In general, fermenters are either agitated by turbines to obtain the required mass transfer or by draft tubes that use the air lift principle. Both types have been tried in this research and each have been found to have advantages and disadvantages. The draft tube type is probably the most desirable for a production plant because of the low power requirements and the minimal maintenance needed. The turbine is best for research and pilot plant work because of its ease of manipulation. The fact that much smaller amounts of oxygen are required for this

fermentation than for yeast grown on hydrocarbons means lower power and maintenance costs.

This requirement of minimal oxygen transfer during fermentation also has another side advantage, that is, since less oxidation is being carried out during the fermentation step, less heat has to be removed to maintain constant temperature for the growing microorganisms. Wang (13) has calculated that the amount of heat released by microbial oxidation of carbohydrate is approximately 5,840 BTU per pound of cells produced, while the exotherm for a microbial oxidation of hydrocarbon is about 14,000 BTU; for methane conversion it is about 33,500 BTU per pound of cells produced. These data are of great importance in large scale production because the relatively low growth temperatures of most microorganisms leaves little driving force in terms of temperature differential between fermenter operating temperature and normal cooling water. Later in the results section it will be shown that because this process uses an alkaline preoxidation step less heat will be liberated than the theoretically low amount of 5,840 BTU calculated by Dr. Wang.

Foam

Aerobic fermentations are invariably accompanied by a large amount of foam generation particularly when undissolved particles are present to act as nucleation centers. Mandels and Weber (14) have reported this difficulty while producing cellulase enzymes on cellulose substrate. Golueke in his 3rd Annual Report to the Bureau of Solid Waste on Biological Fractionation of Solid Waste has shown very dramatically the difficulties associated with foaming while producing cellulase enzymes (15).

In this work we have found that the microorganisms tend to concentrate

in the froth and a distribution coefficient of as high as 10 to 1 in favor of the foam has been found. Of course, the microorganisms cannot be effective if they are being concentrated in the foam and then being removed with the exit air stream or the menstrum overflow. Although nothing tried to date has been completely effective, polyglycol P-2000 has given the best performance. In our case the antifoam agent must be food approved since the end product is aimed at animal feed. This eliminates many of the potential antifoam agents currently in use.

Microorganisms

As mentioned earlier, the cellulose consuming microorganism used in this research was isolated from a sugar cane field in the Baton Rouge area by V. R. Srinivasan and Y.W. Han (16). The organism was found to be an aerobic and mesophilic bacterium, a gram negative rod approximately 1.5μ long and 0.5μ in diameter with no flagella. These investigators used the terminal dilution method to isolate a single clone of cellulolytic organism from related contaminants. These investigators reported that the organism rapidly broke down alkali treated sugarcane bagasse (17).

Subsequent studies have shown that the cellulase enzymes produced by the organisms are probably intramural or bound to the cell walls since repeated efforts to isolate enzymes from the menstrum by precipitation with ammonium sulfate have failed. The enzymes appear not to be constitutive but must be induced. Not only do the enzymes need to be induced but they also appear to be very rapidly repressed by the presence of small soluble carbohydrate molecules that can diffuse through the cell walls of the organism.

Although the evidence are still incomplete, it appears that the organisms

do not have the C_1 enzymes suggested by Reese, Sui and Levinson (5) as being necessary for the complete hydrolysis of cellulose. Although chemical pretreatment appears to be unnecessary for the consumption of cellulose by fungal enzymes such as trichoderma viride, there are indications that such a treatment could greatly aid the rate of digestion (18).

A rather interesting discovery was made during this research when running under non-sterile conditions. Very heavy cell growth was noticed one day when operating conditions were not greatly different than they had been previously. The suspicion of encroachment by a foreign organism later turned out to be correct. The intruder was found to be a bacteria that was very high in Beta-glucosidase activity. It was identified by Han and Srinivasan (19) as being Alcaligenes faecalis.

When subsequent fermentations were inoculated with a mixture of the two cultures, growth rates were greatly enhanced as shown in Figure 1. The reason for such extraordinary growth rates obtained with the symbiotic cultures is somewhat difficult to understand. Originally, it was felt that the second organism served the function of disposing of one of the products of the consecutive reactions that must be involved in the hydrolysis of cellulose down to glucose. Whatever function the Alcaligenes performed, it did greatly increase the rate of cell growth and growth can only come from consumption by the organisms of the products of hydrolysis of the cellulose. Whistler and Smart (20) have shown that a build-up of cellobiose can be expected in the enzymic hydrolysis of cellulose and that little if any other oligomers may be anticipated. Therefore, the function of the second organism is probably removal of the disaccharides which had been the rate controlling step in the series of consecutive reactions.

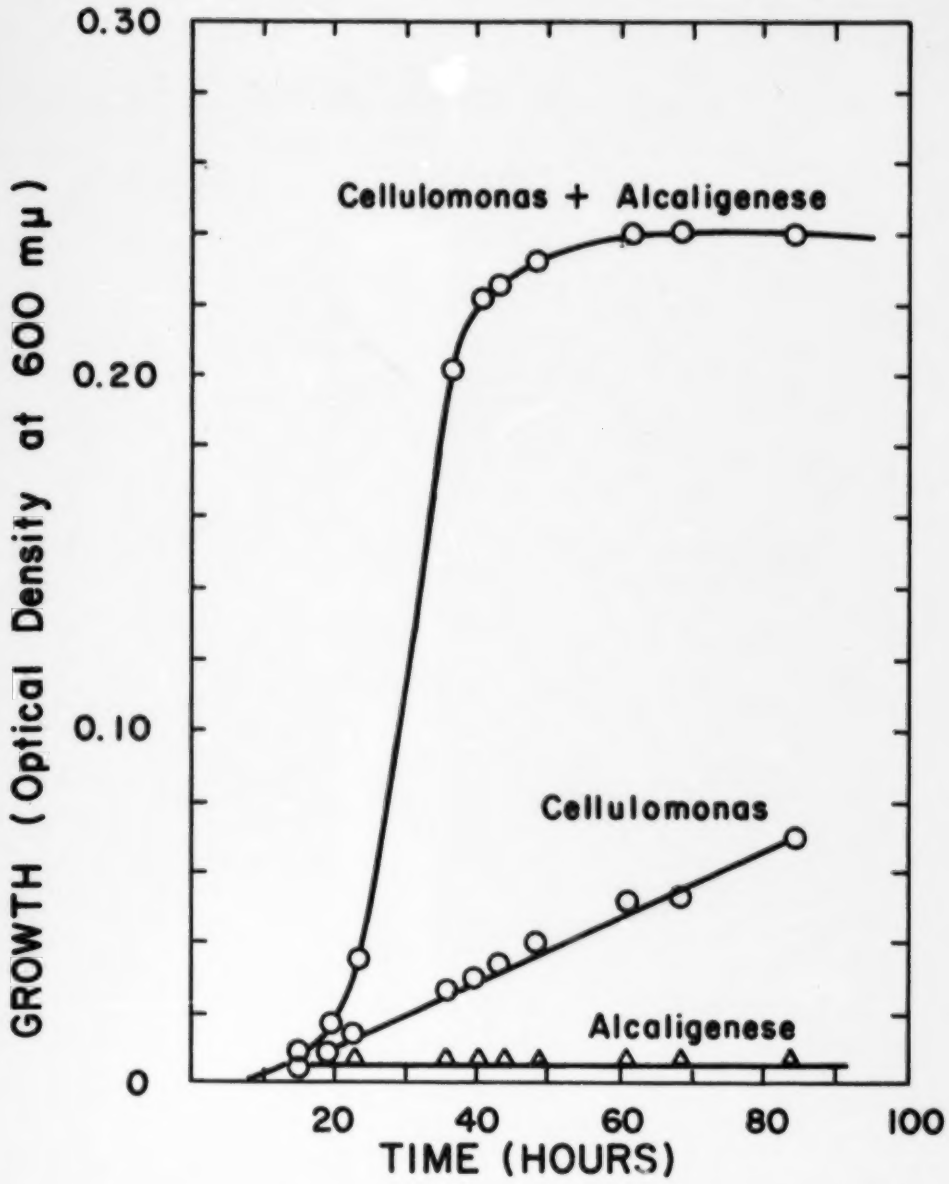


FIGURE 1 The kinetics of growth of Cellulomonas and Alcaligenes in CM-cellulose medium. The organisms were grown separately and were then harvested and washed with physiological saline. One-tenth ml each of cell suspension was inoculated into flasks containing 500 ml of 0.5% CM-cellulose medium. The medium consisted of basal medium supplemented with 0.002% of thiamine. The third flask was inoculated with 0.05 ml each of the two organisms.

*Data from Studies on the Bacteria and Bacterial Enzymes involved in the Degradation of Cellulose by Y. W. Han. A dissertation of Department of Microbiology, L.S.U., 1969.

Recent experiments have shown that perhaps the Alcaligenes also serves to prevent inhibition of the cellulase enzymes once development has occurred. That is, if cellulase activity has once been established, the addition of soluble carbohydrates can cause inhibition in activity of the preinduced cellulase enzymes. From a theoretical standpoint, it would appear that inhibition by disaccharides or cellobiose would be most feasible since the molecules must be small to be able to rapidly diffuse through the cell walls of the growing organisms to cause repression. Molecules smaller than the disaccharides would not enter into the picture because extremely fast growth rates have been shown by both organism growing on glucose and smaller molecules.

Experiments by V. R. Srinivasan and his students in trying to elucidate the mechanism of the two organisms are summarized in Tables III, IV, V, and VI. Here they show the high specific activity of beta-glucosidase from Alcaligenes and a high specific activity of the beta-gluconases from Cellulomonas. In other words the Alcaligenes much prefers the shortest polysaccaharides while Cellulomonas has the reverse preference.

Kinetics of Cell Growth

Mass doubling times and the kinetics of cell growth have been discussed quite thoroughly in our report on the construction of the pilot plant to the Bureau of Solid Waste Management (21) and will not be repeated here. One or two additional points will be brought out that were not emphasized there. First, the basic log phase mass doubling time of the organism or the slope of cell concentration vs time on a semi log plot is extremely important

Table III

Level of B-Glucanase in Organisms Grown on Casein Hydrolysate with Different Carbohydrates

Substrate	units/g. of protein B-glucanase activity
Glucose*	3.1
Cellulose powder	4.4
CMC	4.5
Cellobiose	11.3
Lactose	4.0
Maltose	3.7
Fructose*	3.4
Mannose	3.5
Casein only*	3.1

*Very poor growth

These data were taken from, Pruification & Properties of β-glucanase from a cellulomonas species, M.S. Thesis by R.W. Chang-L.S.U. Department of Microbiology, 1971.

Table IV

Purification of B-Glucanase

Treatment	Total Vol. ml.	Total Prot.	Total Enz. units	Sp. Act.	Enz. Yield
Crude extract	37.0	703	13.4	0.019	100
Precipitate of ammonium sulfate 40-60 percent Sat.^{n}	12.5	115.6	9.3	0.08	69
Sephadex. G-200	180	43.2	7.5	9.175	56
DEAE cellulose	84	14.3	6.0	0.42	45
Ammonium sulfate ppt^{n}	20.7	10.8	8.1	0.75	60

Table V

Hydrolysis of Various Glucosides by the B-Glucanase

Substrate	Glucose Liberated mg/ml
Cellobiose	0.13
Cellotriose	0.16
Cellotetraose	0.19
Sucrose	0.015
Lactose	0.00
B-methylglucoside	0.015
Salicin	0.06

Table VI

Comparison of the Properties of the Enzymes from Cellulomonas and Alcaligenes

Factors	Cellulomonas	Alcaligenes
Relative rates of hydrolysis	Tetra>Tri>di	Tetra<Tri<di
Mol. wt.	250,000	150,000
Optimum pH	5.5	6.0-7.0
K_m (M) for PNPG	3.6×10^{-4}	12.5×10^{-5}
Enzyme formation with cellobiose	Inducible	Inducible
with CMC	Inducible	Non-inducible
with Lactose	Non-inducible	Inducible
K_i B-methyl glucoside (M)		5.6×10^{-2}
K_i cellobiose (M)		1×10^{-7}

because of its effect on the volumetric production efficiency (VPE). It is quite obvious that the steeper this slope the more bio-mass is being produced per unit of time and the smaller may be the fermenter to produce a given amount of protein. We have observed values of the mass doubling time as short as 1.5 hours and as high as 6 hours.

Considerable effort has been expended in this research on methods to increase this slope. The shortest doubling times have been noticed when the microorganisms are growing on a soluble substrate such as lactose while the longer times are observed for cells growing on cellulose with a minimal amount of pretreatment. There is apparently some relationship between the growth rate and the degree of crystallinity of the cellulose. Several factors in the chemical pretreatment appear to have an effect on the final degree of crystallinity of the cellulose. These are caustic concentration, temperature of treatment, time of treatment, oxygen partial pressure, concentration of oxidation catalysts, and concentration of oxdizing agents. Although their effects on growth rate may be determined each variable will have a different influence on the overall economics of the process.

Regardless of the effect of various substrate pretreatments, consumption of the insoluble cellulose by these bacteria are extremely fast in comparison to the rates obtained by other investigators using fungi (11, 14, 15). In our experiments, digestion occurs in a matter of hours rather than days.

It is true that an exhaustive hydrolysis of the cellulose, such as that performed by the Germans during World War II, in which the cellulose is completely converted to soluble simple sugars, would give fast growth rates

of almost any organism on the hydrolysate (22, 23). However, the cost of the hydrolysis step appears to place such an economic load on the process that it is non-competitive. One can assume this latter statement to be true since no commercial processes are in operation at the present time to convert cellulose to yeast protein. At least one commercial plant is in operation to produce yeast from solubilized wood sugars where the hydrolysate comes as a by-product of a pulping operation. Meller (22) in his well documented report to the Bureau of Solid Waste has shown clearly that a process requiring complete pre-hydrolysis with acids could not be competitive with soy bean protein when the starting material is unhydrolyzed cellulose.

Cell Concentrations

Not only does the mass doubling time have an effect on the economics but an equally important role is attributed to the cell concentrations. The cell concentration divided by the retention time gives the volumetric production efficiency. Klass (24) has shown the relationship between capital investment, doubling times and cell concentrations for growing SCP on methane. His data show that for cell concentrations below about 3 grams per liter, the capital investment increases asymptotically with further decreases in concentration and that above about 7 grams per liter the capital investment approaches a limiting minimum such that increases above about 7 g/l will have only minor effects on the total investment assuming mass doubling times in the order of 1 to 7 hours.

To put it another way, increases in volumetric production efficiencies above about 0.75 grams per liter per hour have little effect on the total capital investment for a plant producing SCP from methane. Admittedly, this

comparison involves two completely different substrates so that the base cost may not be directly comparable, still the changes will be comparable.

Cell Harvest

The effluent coming from the fermenters contains unreacted cellulose, unused nutrients, salt, lignin, cells and small amounts of soluble carbohydrates. Before going to cell harvest, the cellulose must be removed from the suspension for recycle. This step is not simple because if the filter has large openings the cellulose will go through, while small openings tend to plug and hold back part of the cells.

Limited experiments have shown that when the pH of the effluent is reduced to 4.5 or 5.0, a large part of the lignin becomes insolubilized and floats to the surface. A large fraction of the unconsumed cellulose also floats and could be removed with a skimmer. This mixture of lignin and unreacted cellulose could not be recycled to any great extent in a mixture so high in lignin because of recycle build-up of lignin. Furthermore, not all of the cellulose comes to the surface with acidification. The cellulose floatation appears to result from residual cell activity which generates gaseous metabolic by-products while consuming some of the remaining cellulose particles. The gas in turn becomes trapped on the porous surfaces of the particles causing floatation. Since large settling vessels are somewhat difficult to keep sterile, too much fermentation in this stage could lead to product contamination. Microbial activity is, however, greatly reduced by this large drop in pH.

Generally speaking, acidification of the effluent causes the cells to floc and precipitation to start. Cells were concentrated in this manner before feeding to the drum dryer for several pilot plant runs. Unfortunately, these cells contained large quantities of ash, which in some cases ran as high as 25% of

the cell weight. This was due to the formation of salt in the neutralization process which remained with the cells after subsequent neutralization and drying.

Unreacted Cellulose

Only minor effort has been expended to date in attempting to obtain filter designs for removing the unreacted cellulose from the fermenter effluent for recycle. The feeling has been that without high volumetric production efficiencies, there would be little need to develop this step. Recent experiments have shown, however, that the process is becoming competitive so more effort will be expended in this area in the future.

At the present time, a fine wire screen is used to filter out a large portion of the unreacted cellulose, but because some of the cellulose particles are extremely small, not all of the cellulose is removed. If the cells are harvested by a solid bowl centrifuge, this unit can then be used to remove the remainder of the cellulose. The small cellulose particles are thrown out very near the liquid entrance to the bowl and can be physically separated from the cells which come out further down the bowl passageway.

The best method found to harvest the cells has been by the use of flocculants. Both cationics and anionics have been used. The best results have generally been found with polyethyleneimine in concentration ranges as low as .05 to .01 grams per gram of cells. Some difficulty has been experienced in finding a sufficient variety of flocculents to test which have FDA approval.

A rather interesting relationship has been found between cell age and flocculent requirement. It appears that the longer the cells are held in storage before flocculation, the more flocculent is required. This phenomena has also been observed by McGregor and Finn (25). They have shown that fresh

cells from the fermenter start to slowly leak autohydrolyzed RNA which clings to the surface of the cells. The presence of these nucleic acid fragments on the surfaces cause inactivation of the flocculent with the result that more flocculent has to be used to obtain the desired amount of settling.

They found that the cure for this phenomena was to submit the cells to vigorous agitation just prior to addition of the flocculent. This treatment apparently washes off the RNA and proteins retained on the surfaces such that only one tenth as much flocculent was required to settle cells that had aged several hours. This treatment is obviously a step that will be included in the pilot plant as soon as time and money permit.

The diameter of cellulomonas is approximately 0.5 microns while its length is about 1.5 microns. The Alcaligenes which is grown symbiotically in mixed culture is about 1.0 micron in diameter and perhaps 2.5 microns long. These bacterial cells are extremely small when compared to yeast at 6 to 8 microns. Desludging centrifuges have been found to be very effective in harvesting yeast cells. However, the power requirements for sedimentation are proportional to the reciprocal of the particle diameter squared. If the particle diameter were one half that of yeast, four times as much centrifugal force would be needed to effect sedimentation. Since cellulomonas is even less than one half the diameter of yeast, it means that the power requirements for centrifugal separation are probably much too high to offer a practical means of concentrating the cells.

Analytical Techniques

Earlier in this report it was mentioned that considerably difficulty has been encountered in obtaining precise analytical data. In order to obtain cell concentrations as a function of time, turbidity is usually

measured and the amount of light adsorbed or transmitted is then proportional to the number of cells present. The basic premise of this technique is that there are no other strong light adsorbers present in the menstrum nor are there any other small particles present to scatter the light. When bagasse is used as the feed, neither of these two basic assumptions are correct. The lignin is a very strong adsorber at any wavelength used. Its color, and therefore its adsorbance varies with the conditions of alkali treatment; the concentration of the caustic used, as well as the temperature and time of alkali treatment.

In order to eliminate this undesirable effect of the change in adsorption by the lignin with treatment history, an effort was made to run turbidity on the sample, spin out the cells and then rerun the turbidity. The difference between these two readings should then be related to cell concentration. Unfortunately, this also gives incorrect answers because of the presence of very very small cellulose particles that remain in the sample after the initial filtering step used in preparing the samples. Any attempts to first remove these extremely small cellulose particles ends up in the removal of part of the cells. A large part of our research efforts are allocated to finding the answer to this particular problem.

Attempts were also made to use other methods of determining cell concentrations such as the Lowry (26) and the Biuret tests (27) which are based on determining the amount of protein present. These methods also gave extremely poor results. Finally the ninhydrin test (28) has been found to give better results but this procedure requires hydrolysis with 6N Hcl at 146°C in sealed tubes under a nitrogen atmosphere for four hours. The relative value of these tests for protein determination is summarized in a paper by Daly and Ruiz (29).

Obviously cell concentrations are not the only analytical data needed. A portion of the initial cellulose is solubilized by the alkali pretreatment. The conversion of this soluble carbohydrate to protein occurs with a yield of

approximately 50%. The conversion of solid substrate to protein occurs with about 40% yield. It would be highly desirable to know how much soluble carbohydrate is present at any time in order to obtain a reliable material balance. The phenol-sulfuric acid test (30) has been used for this determination for some time. Unfortunately, this technique which measures glucose concentrations quite accurately, does poorly on larger sugar molecules and is extremely poor on oligomers greater than 4 to 5 anhydroglucose units long. Poor accuracy is also obtained with the DNS method (31).

Some investigators merely run a material balance around the process by weighing in a known amount of solids and then recovering the remaining solids after the experiment is over (11,32). This may be a good practice if all that is desired is volume reduction of the cellulosic waste, however, the liquid effluent must end up in a stream sooner or later which may in effect just transfer the problem from one type of pollution to another. Recent experiments using recoverable solids as a function of time, along with the ninhydrin test for cell concentrations and DNS test for soluble carbohydrates has given material balances that check within 5-10 percent.

The best way to follow cell growth as a function of time for laboratory and pilot plant fermenters that have pH controllers is to follow the amount of NaOH required to maintain the pH constant throughout the run. This technique suggested by Dr. Wang (33) is quite precise if the pH control is precise. Of course, it does not work with shake flasks since here the pH is controlled by a buffer system.

Product Utility

Any scheme for the recycle of solid waste must meet several criteria, any of which if overlooked, can mean failure for the project. Perhaps the first

and most important requirement is product utility. Because of the staggering amount of solid waste, the product that is produced must fulfill a real need in the economy as it currently exists. In other words, if millions of tons are to be processed for recycle there must be a ready market for millions of tons of the product. Very few proposed products meet this requirement.

Perhaps the best example of this is compost. Zaltzman and Tarquinio (34) in their literature review of the biological decomposition of cellulose have many many references to composting. Compost Science, a bi-monthly periodical published by Rodale Press summarizes the current progress in this field. The product that is produced in 7 to 10 days of aerobic decay has little if any value and attempts to sell it for a penny or two a pound as a soil aeration aid have met with little success.

Even less success is obtained by using anaerobic digestion of waste cellulose in the presence of activated sludge. Here long residence times of 15 to 30 days (11) are required to produce a product which at the present time requires costly incineration for disposal.

No one need be reminded of the current shortage of protein that exists in the world today and with the population explosion that is occurring things can only get worse. It would appear, then, that not only is there an extremely large ready market for protein if it could be produced economically for recycle but also production of such a product would help solve this second very real problem.

Many attempts have been made to develop new foods and force these foods into the diets of indigeneous populations. Perhaps the best summary of such efforts has been proposed by Dr. Felicia Pryor (35) who said "Food is not a food until it has been eaten". Recent developments in food and nutrition research have shown that food may now be considered as a series of chemical

compounds, each required in varying amounts which can change with changes in one's environment, exercise, living habits, etc.

One of the basic elements required by all living systems is a nitrogen supply. For ruminants this may come merely in the form of fixed organic nitrogen such as urea. For humans, however, the nitrogen source must be in the form of balanced amino acids. Generally, the utility of a protein source for humans is limited by the presence of that essential amino acid present in the smallest amount.

The best nitrogen source then is one which comes as proteins which contains a proper balance of the essential amino acids (36). Although the correct ratio of these essential amino acids in the diet is not known precisely, FAO has suggested an ideal amino acid pattern as shown in Table VII.

Table VII also shows the essential amino acid content of cellulomonas and the profile of those products which might conceivably be competitors to the product produced here.

Cellulomonas has as its limiting amino acid, methionine at 2.16%. When this product was fed to male wealing rats as the sole source of nitrogen for 10 days, the rats showed a weight gain if fed at the 40% level. If supplemented with 0.3% methionine, weight gains were shown at the 10% intake level. One very important observation from these data was that no toxic effects whatever were found with rats fed Cellulomonas at a level of 60% of the diet for 10 days.

One unexpected and very fortunate result of the change to symbiotic cultures has been the great improvement in both the digestibility and the amino acid profile of the new product. Table VII A shows the essential amino acid profile for pure Alcaligenes and the mixed culture. The data on Cellulomonas and the FAO reference protein have been repeated in this table for ease of comparison. Also included are data on egg protein often considered the most

TABLE VII

ESSENTIAL AMINO ACID CONTENT OF THE SINGLE CELL PROTEINS PRODUCED AT LSU AND OTHER LABORATORIES

Amount (g/100 g protein)

Essential Amino Acid	Pure (1) Cellulomonas	ESSO(2)	British Petroleum(3)	Torula Yeast(4)	Ideal Amino Acid Pattern by FAO (2)	Soybean Protein(2)
Arginine	7.43	*	*	*	*	7.0
Histidine	2.91	*	*	2.2	*	2.5
Isoleucine	4.46	3.6	5.3	6.4	4.2	5.8
Leucine	9.76	5.6	8.1	8.0	4.8	7.6
Lysine	7.87	6.5	7.6	8.5	4.2	6.6
Methionine	2.16	2.0	1.7	1.5	2.2	1.1
Phenylalanine	4.26	2.9	5.7	5.1	2.8	4.8
Threonine	5.20	4.0	5.8	5.1	2.8	3.9
Valine	7.3	4.5	5.7	5.6	4.2	5.2
Tyrosine		*	*	4.3	2.8	3.2
Half Cystine		0.6	1.0	1.0	2.0	1.2
Total S Amino Acids		2.6	2.7	2.5	4.2	2.3
Total Aromatic Amino Acids		2.9+	5.7+	9.4	5.6	8.0

(1) The sample was hydrolyzed with 6 N Hcl at 110°C for 22 hours and determined with a Beckman Model 116 amino acid analyzer.

(2) Chemical and Engineering News, P. 47 (Jan. 9, 1967).

(3) Personal Communication to S. P.Yang.

(4) Lake State Division, St. Regis Paper Company, Rhinelander, Wisconsin.

(5) R. J. Block and K. W. Weiss, Amino Acid Handbook, (1956).

* Not reported or specified.

TABLE VIIA

ESSENTIAL AMINO ACID PROFILE OF LSU-SCP IN COMPARISON TO REFERENCE PROTEINS (Grams AA./100 Grams Protein)

Amino Acid	Cellulomonas	Alcaligenes	Symbiotic	FAO REF.	Egg
Lysine	7.87	6.57	6.87	4.20	6.30
Histidine	2.91	2.81	3.03	-	-
Arginine	7.43	8.01	9.18	-	-
Threonine	5.20	4.89	4.73	2.80	5.00
Valine	7.30	7.84	7.59	4.20	7.40
Methionine	2.16	2.93	2.50	2.20	3.10
Isoleucine	4.46	5.59	4.92	4.20	6.80
Leucine	9.76	8.61	8.36	4.80	9.00
Phenylalanine	4.26	4.89	4.42	2.80	6.00

digestible food available.

Table VII B shows the results of feeding studies obtained by Dr. W. F. McKnight of our Home Economics Department. This table also lists the protein efficiency ratios and the nitrogen digestibilities obtained feeding those diets listed in the table. The results represent averages for 8 rats on each diet for ten days. The nitrogen digestibility was obtained by a material balance around the rats after determining fecal nitrogen.

TABLE VIIB

RESULTS OF FEEDING TRIALS WITH LSU-SCP RUN #19 & 20

Diet Description	Weight Change gms.	Food Consumed gms.	PER	Nitrogen Digestibility %
1. Protein Free	- 8.0	60.3	-	-
2. 10% Casein	27.9	96.8	2.88	84.0
3. 20% Casein	59.6	119.6	2.91	91.0
4. 20% Alcaligenes	30.1	94.2	2.23	77.5
5. 40% Alcaligenes	52.6	93.2	1.97	73.8
6. 80% Alcaligenes	-0.9	46.1	-	64.9
7. 30% Symbiotic Freeze Dried	45.3	90.5	2.46	72.2
8. 30% Symbiotic Drum Dried	41.9	94.2	2.21	76.5

Srinivasan and Han (17) have presented more data on the comparison of the Cellulomonas protein with other foods.

Results

It has been shown in the preceding sections that the protein product produced in this research does have utility in the feed market almost immediately and may have utility in the human food market in the future (37). Utility by itself, however, is not a sufficient criteria on which to base potential sales. This can only come through a combination of utility and competitive production costs. This section of the report will attempt to present the economics of the process as it now stands based on actual operating data obtained in the pilot plant. A large part of this data has already been recorded in our report SW-24C to the Bureau of Solid Waste, most of the remainder will be reported in a second article on the pilot plant operation. Therefore it will only be summarized here.

Because it is generally easier for the reader to evaluate cost factors by comparison with similar steps of other well developed processes, an effort will be made here to present comparative data from other sources. As already pointed out earlier in the report the chief competitor for the single cell protein market is yeast proteins grown on hydrocarbons. Here a process has been developed and at least two large scale production units are already in operation. One of these plants is owned and operated by British Petroleum and it would be more accurate to use their detailed cost figures for comparison. Unfortunately, these are hard to find but Bennett, et al. (1) have given the overall economics. The French Petroleum Institute (IFP) claim to have nearly an identical process to BP's except that they produce candida yeast rather than torula and they have reported a large amount of cost data (38). Therefore, IFP data will generally be used in this report for comparative purposes. Wang (39) has also presented an overall economic analysis for yeast

production on hydrocarbons. Takashi (5) reports that Kanegafuchi Chemical of Japan is producing more than 2 million lbs/month of Saccharomycea yeast for animal feeds but gives little details of the design of the plant nor any cost data.

Meller (22) has presented an economic analysis of yeast production on acid hydrolyzed cellulose. Although this process appears not to be competitive, the design presented in the report is quite detailed. Goleuke (15) presents a rough estimate of the economics of a process where waste paper is enzymatically hydrolyzed to glucose with the enzymes from Trichoderma viride QM 6A and the glucose produced is subsequently used as a substrate for fungal protein production. He reports the theoretical possibility of producing glucose at a little over seven cents a pount. He also points out that the analysis is subject to a great deal of uncertainty. It isn't difficult to agree with this conclusion.

Raw Material

The raw material generally used in this process has been bagasse, which has a fuel replacement value of 0.25 cents per pound. The pilot plant yields of cells on this rather poor source of cellulose are about 0.35 pounds per pound of feed or 2.86 lbs of bagasse must be fed to produce a pound of cells. This means a bagasse cost of 0.72 cents per pound of cells. The yield values are somewhat higher if the source of cellulose is more pure as would be the case for newspapers, writing paper, books, magazines, etc.

Boettcher (40) has shown that recovery of 25% of the cellulose in urban solid waste using air classification would give mixed paper at $5.00 per ton. Chemical Engineering of June 21, 1971 shows a flow sheet on page 159 that can be used to obtain the cellulosic portion of urban solid waste. This report shows that the cellulose could be recovered from urban solid waste at a cost

about comparable with the fuel replacement value of bagasse. If one does as Meller (22) suggests and takes a dumping credit at $4.50 per ton, this would approximately offset the cost of air classification and would give the feed at essentially no cost. The nice part of this approach is that slightly better yields would be obtained on urban cellulosic waste so that not so many pounds of raw materials are necessary to produce a pound of product.

The cellulose feed must be ground before being treated with alkali. With bagasse, a knife grinder can be used that will grind more than 4,600 pounds per hour through a 1/8 in. screen using a 100 HP motor. Considerable effort has been expended in this research to see if there would be any advantage to grinding to smaller particle sizes. Virtanen and Nikkila (41) report that the rate of cellulose fermentation increases with an increase in the fineness of wood flour. All experiments in our laboratory have indicated very little increase in the rate of digestion with a decrease in particle size down to about 50 microns. Power requirements are inversely proportioned to the particle diameter squared showing therefore that a very large increase in power is required for a small decrease in particle size.

Alkali Treatment

Native cellulose requires a chemical pretreatment for rapid consumption by these organisms. One of the purposes of the alkali treatment is to disrupt the crystallinity of the cellulose. Crystallinity appears to play an important role in the rate of cellulose digestion, and many investigators have concluded that microbial attack can occur only in the amorphous regions. This appears to be true, and the paper that this is written on would digest but only to a limited extent because even though crystallinity was disrupted during a pulping operation, it was reestablished during the drying step.

Support of the theory that rapid microbial attack occurs only in the amorphous regions is shown by experiments that were run in the laboratory

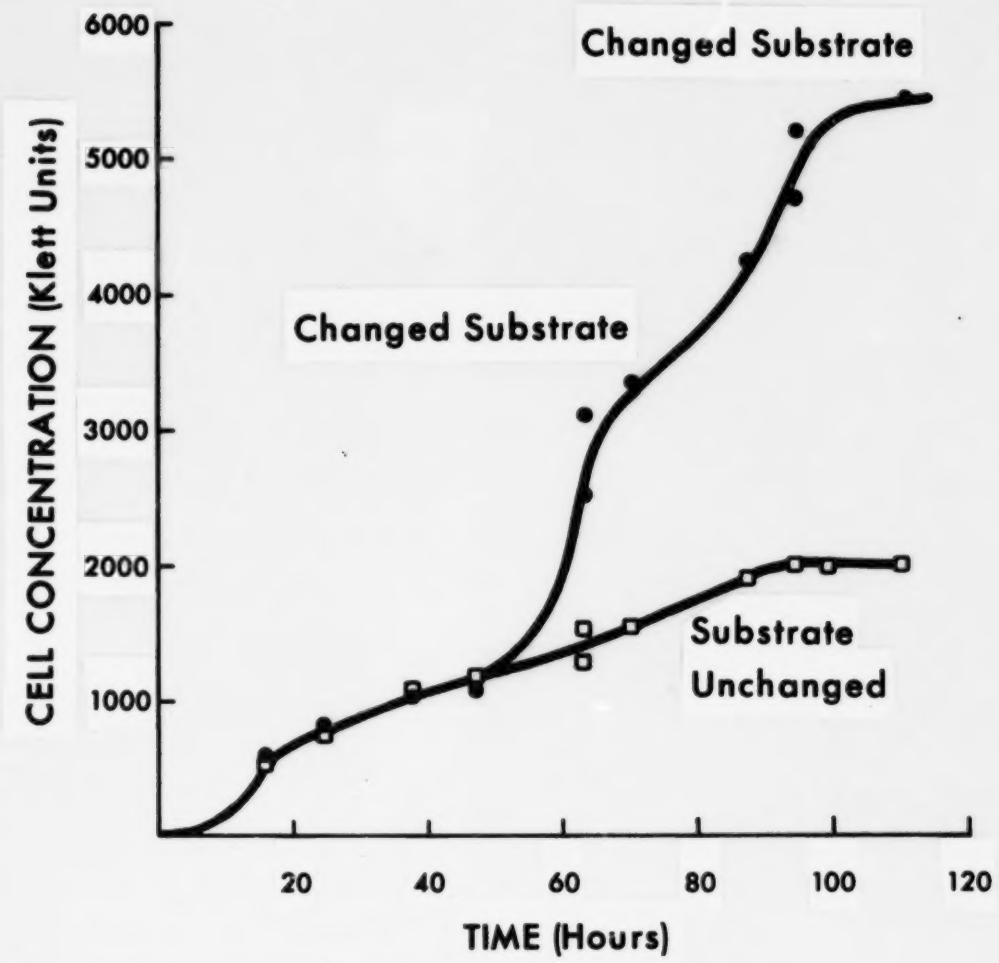
to replace the unreacted, and supposedly crystalline cellulose remaining after partial microbial consumption with more freshly treated bagasse. These data presented in Figure 1A show that removal of the unreacted cellulose, followed by replacement with fresh bagasse greatly increased both the rate of cell growth and the final cell concentration. Cellulose that has been purified and dried can be consumed but to a lesser extent and at a slower rate than moist alkali treated bagasse. Bagasse that has been freshly treated supports growth at a slower rate than does alkali treated bagasse that has aged in air for several days before neutralization. Extremely rapid growth rates are obtained with lactose, glucose, glycerine, BHI, etc. as shown in Figure 1B. In summary, the more exhaustive the alkali treatment or the more soluble carbohydrates the more rapid the microbial growth rates.

Oxidation does more, however, than just lower the degree of polymerization of the cellulose substrate. It also causes disruption of the crystallinity of the cellulose molecules by inserting bulky oxidation residues such as carboxyl groups between the cellulose chains preventing their return to a close packing lattice arrangement.

At least 5 independent variables have an influence on the microbial growth rate obtained after a given alkali treatment. These are as follows:

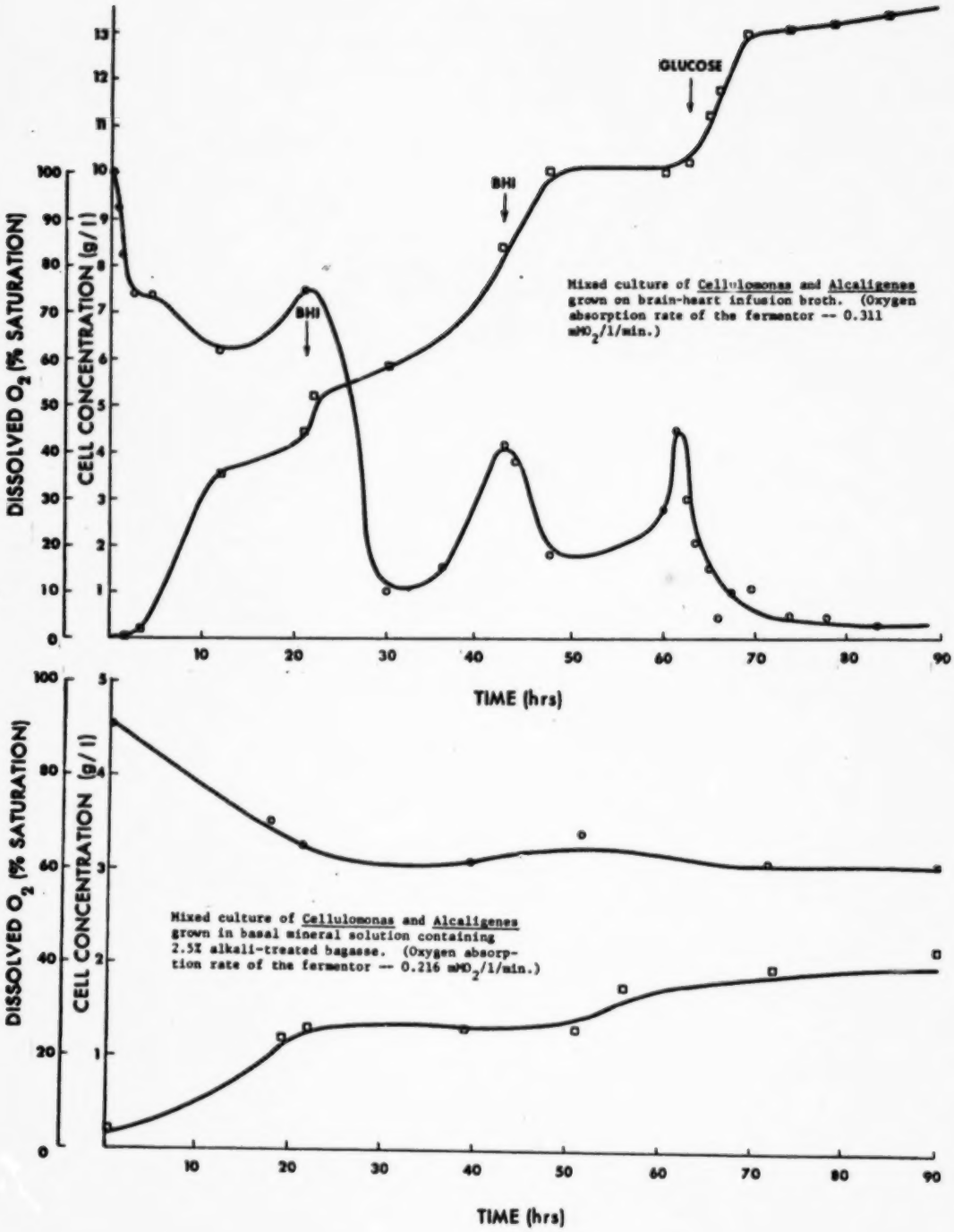
1. Caustic concentration
2. Temperature during caustic treatment
3. Oxygen partial pressure during treatment
4. Time of treatment
5. Concentration of oxidizing catalysts $CoCl_2$ $SnCl_2$, NaOCl, etc.

Figure 1C shows the total cell growth after 118 hours as a function of both the caustic concentration and the temperature at which the treatment was carried out. These rather limited data points show that caustic concentration is more important at low temperature than it is at high temperature. These data are based on treatment times of 5 and 10 minutes and assumes a negligible effect due to the differences in time.



Mixed culture of Cellumonas and Alcaligenes in basal mineral solution containing 2.8% alkali-treated bagasse. (Oxygen absorption rate of fermentor:0.216 mMO_2/l/min.)

FIGURE 1A
KINETICS OF GROWTH
WITH PERIODIC REPLACEMENT
OF UNCONSUMED BAGASSE


GLUCOSE
BHI
BHI
Mixed culture of Cellulomonas and Alcaligenes grown on brain-heart infusion broth. (Oxygen absorption rate of the fermentor -- 0.311 mMO2/l/min.)
DISSOLVED O2 (% SATURATION)
CELL CONCENTRATION (g/l)
TIME (hrs)
Mixed culture of Cellulomonas and Alcaligenes grown in basal mineral solution containing 2.5% alkali-treated bagasse. (Oxygen absorption rate of the fermentor -- 0.216 mMO2/l/min.)
DISSOLVED O2 (% SATURATION)
CELL CONCENTRATION (g/l)
TIME (hrs)

KINETICS OF GROWTH and DISSOLVED OXYGEN CONTENT

FIGURE 1B

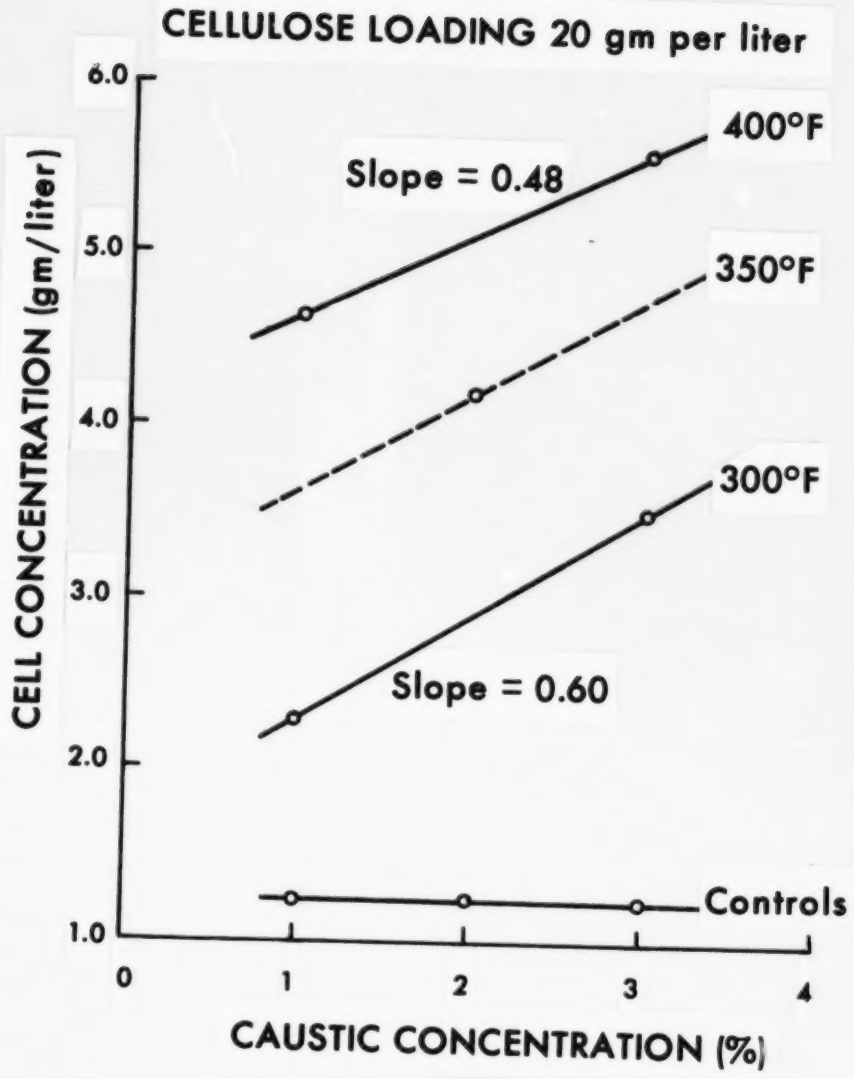


FIGURE 1C

EFFECT OF PRETREATMENT ON CELLULOSE DIGESTION

Within limits, an increase in any of these variables causes an increase in the rate of digestion and usually an increase in the extent of digestion. Each change in a variable also carries with it an economic penalty on the process and each is different. If the precise effect that each variable has on the growth rate were known along with its cost then it would be possible to predict the optimum conditions under which to operate.

A statistically designed set of experiments was run using a one half factorial with two levels of each variable listed above except catalyst concentration. This was held constant because tolerance by the organisms limited its concentration to 100 ppm in the caustic solution.

The data were analyzed by regression analysis and the following equation was obtained for that case where the caustic contained 100 ppm of cobalt chloride.

$$Y = -4.38 + 0.0197X_1 + 0.0825X_2 + 0.466X_4$$

where

			Level of Variables all low	Level of Variables all high
X_1	=	temperature (300°F - 400°F)	5.91	7.90
X_2	=	time (5 to 10 minutes)	.410	.825
X_4	=	NaoH concentration (1.0 to 3.0 percent)	.466	1.40
		Y =	2.406	5.745
y	=	cell concentration at 118 hours		

The term for oxygen pressure fell out of the equation apparently because the range was too small for it to have a significant effect.

Caustic Concentration

The equation obtained is not too far from that which might be expected. The large effect of caustic concentration has been anticipated as necessary to obtain high cell concentrations. However, the economic penalty for increasing this variable is particularly severe. Sodium hydroxide is 4.7 cents a pound based on the solids, but for every mole of caustic used a mole of acid

at 1.3 cents per pound must be added to neutralize it, furthermore, this causes the formation of salt. High salt concentration would prevent recycle of the menstrum once the cells have been harvested.

Even this does not tell the whole story because the higher the caustic concentration the larger will be the amount of caustic solution retained by the cellulose after the squeezing step. It is obviously very important then to have as good a squeezing process as possible to help combat the adverse effect caustic concentration will have on the overall economics.

Under normal squeezing conditions, the bagasse retains about 65% of the caustic solution when the caustic concentration is 3%. Using the newly installed "apple squeezer", this can be lowered slightly to about 60%. A unit specifically designed for continuously applying high pressure to the solids in a slurry is a French Oil Mill. Designed originally for expressing the oil from cotton seeds, it has found utility in many other areas of chemical processing. Any large scale operation would probably want to have such a unit in the production train. Based on data obtained from squeezing juice from sugar cane with such a unit, one might anticipate that the retained caustic solution would drop from 60% to about 42% or a decrease from 1.5 pounds of caustic solution per pound of bagasse to 0.725 pounds per pound of bagasse. This represents a decrease in caustic and HCl costs from 0.27 cents per pound of bagasse to 0.18 cents per pound; a significant reduction. If one decides not to change equipment, then a cost of approximately 0.09 cents per pound of bagasse per percent caustic concentration can be anticipated.

Temperature Effects

Generally any increase in the temperature during caustic treatment will result in enhanced microbial growth rates. There is a limit, however, to which this temperature may go if oxygen is present. At elevated temperatures, charring occurs from rapid oxidation. The economic penalty assigned to the temperature variable includes not only the cost of thermal energy for heating the caustic

solution and bagasse but also may include the cost of cooling this stream back down if the exit stream is to be cascaded more or less directly to the fermenter. If separate costs are to be assigned to the sterilization step then the heat load can be assumed to start at 230°F.

Based on a treatment temperature of 400°F with natural gas heat at 40 cents per 1000 SCF (1 million BTU's) the heating costs would be 0.014 cents per pound of bagasse treated. Using river water at 5 cents per 1000 gallons and allowing 20°F temperature raise, cooling costs would be 0.02 cents per pound of bagasse. This can be roughly summarized to equal 2.0×10^{-4} cents per pound per degree over the range tested.

Time of Treatment

It is extremely difficult to assign a precise cost to time since it would depend on what type of holding container was actually used in the plant. There are no utilities consumption associated with holding times unless aeration is introduced to promote oxidation, however, if this were done the aeration costs should properly be assigned to oxygen pressure. Therefore, the only costs anticipated would be the capital costs for the holding vessel itself. Such a vessel could be a silo or it could be a live-bottom-hopper. Live-bottom-hoppers are somewhat more expensive than silos equipped with air conveyors. For a live bottom hopper using a pay out time of 3 years, the cost should be about 0.066 cents per pound per hour. This cost should decrease with increase in size at about the normal 0.6 slope.

Oxygen Partial Pressure

Oxygen partial pressure really refers to the partial pressure of oxygen at the active site where oxidation is to occur. For example, if the bagasse is immersed in excess caustic solution, oxygen from the air must dissolve in

the liquid and then diffuse to the active cellulose surface. This is a rather slow process which depends upon a series of driving forces operating at near equilibrium conditions. Only minor oxidative effects have been observed in alkaline slurries. On the other hand, if the excess liquid is first expressed, oxygen can easily make contact with active sites on the cellulose. If oxidation is to be carried out then it should be done after the alkali treatment but before neutralization.

The conclusion can also be reached that the more caustic that is squeezed from the cellulose, the more rapid will be the oxidation at any given temperature and partial oxygen pressure. The cost to assign to this step would be minimal in open storage of relatively thin layers say 10-12 inches in depth. Perhaps the best system would be that previously mentioned in the June 21, 1971 issue of Chemical Engineering, in the article on Solid Waste Disposal. On page 159, a schematic diagram is presented to show how paper could be separated from urban solid waste for composting. A caustic spray could be inserted into the first shredder and the digester would contain alkali cellulose. Coming from this proposed digester the oxidized cellulose could be slurried in water, neutralized, nutrients and fertilizers added, pumped to the sterilizers, and then on the fermenters.

In summarizing the cost of those variables that effect the extent of conversion, it can be stated roughly that time and temperature increases will cost about the same to give comparable increases in conversion, while the cost of obtaining the same increase by changing the caustic concentration will be larger by a factor of nearly 20.

Sterilization

The necessity for sterilization will depend on how severe the caustic treatment is. If 3% caustic is used and a French Oil Mill to express the excess caustic a significant temperature increase will occur in the screw press. This will essentially complete sterilization of the bagasse. The nutrient salts, recycle liquid, trace minerals, yeast extract, dilution liquid, etc. will still have to be sterilized. However, it is much easier to pump these

liquid components through a plug-flow heat exchanger than it is to meter and pump a cellulose slurry. Any future plants probably should take advantage of this fact.

Sterilization, if required, will be the cost of the steam necessary to raise the temperature of the slurry feed to the fermenter to 240°F, hold it there 5 minutes and then cool it back down to fermenter temperature. If a cross-over-exchanger is used to bring the temperature to 200°F, and live steam injected at 60 cents per 1000 lbs to increase the temperature to 240°F, steam costs would be 0.06 cents per pound of bagasse fed. Cooling costs will be essentially nothing. Such high temperatures and long residence times during sterilization would not be necessary if the caustic is mixed with the entire feed stream before going to the sterilization unit as shown by Han et al (10).

Fertilizers and Nutrients

Recent experiments at the pilot plant that were run with limiting amounts of nitrogen have shown that the microorganisms will consume up to 90% of that amount contained in the feed. From these data it would be safe to assume that a 10% excess of all the required fertilizers and nutrients would be sufficient but may not be necessary if recycle of the menstrum can be established or more complete consumption can be found in later experiments at the pilot plant. Meller (22) in his detailed report on the economics of yeast production from waste cellulose has not considered it necessary to add excess nutrients. Since our experimental data have shown only 90% conversion, the calculations here will be based on this number.

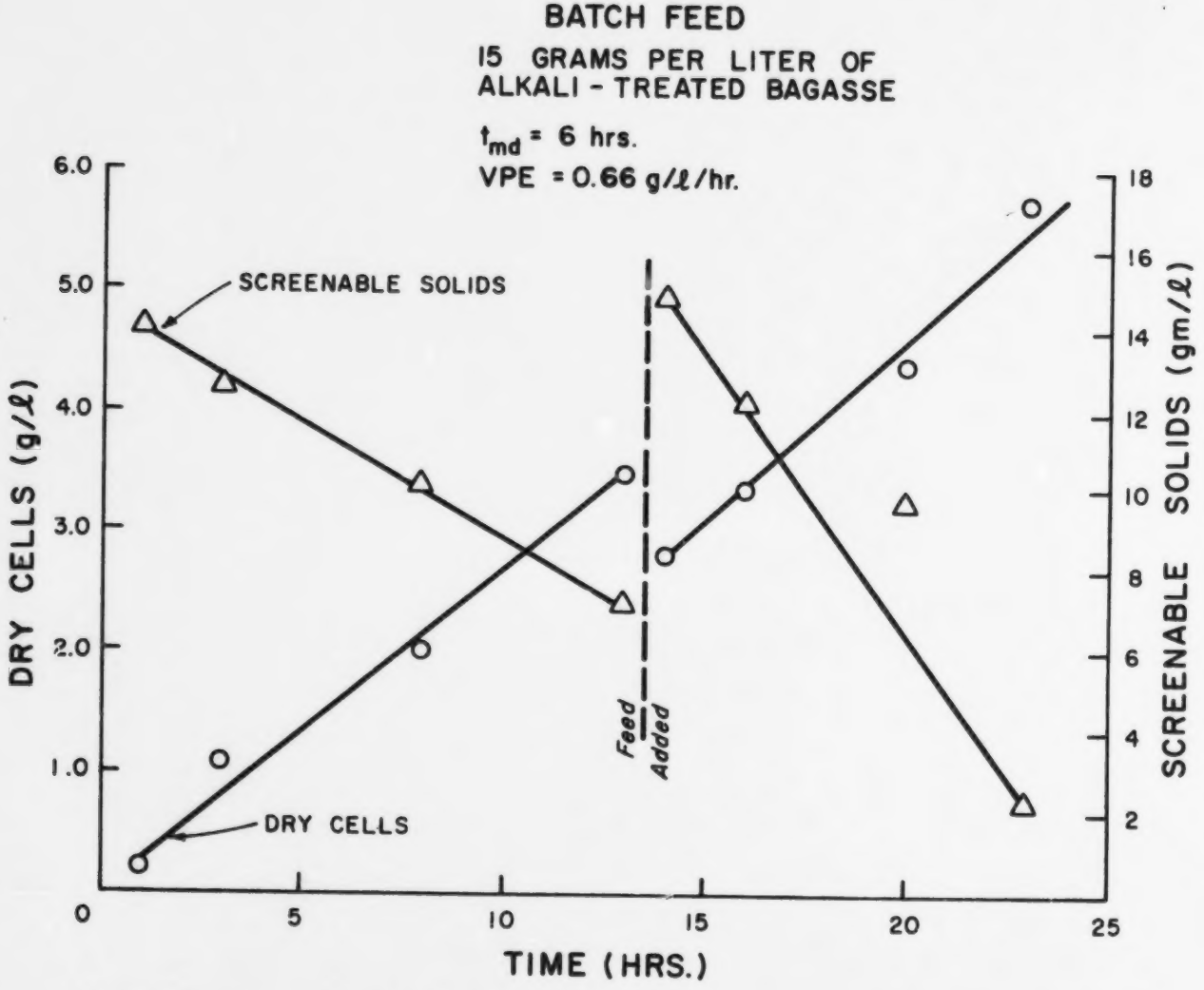
Some mixed cells have been found by Dr. McKnight to contain an average of 55% protein. Based on this, the nitrogen content in the product should be 55/6.25 = 8.8% nitrogen. At 10% excess, this would be 0.097 grams of nitrogen

required per gram of cells. Considerable research has been done to determine the correct or best ratio of nitrogen to phosphorous. This optimum has been found to be about 8 to 1. The amount required then, still using the 10% excess figure is 0.0121 grams per gram of cells. Potassium is also required at about 2.3% of the cell mass weight so the feed should contain 0.0256 gms per gram of cells. Since caustic is required for pretreatment anyway this could be added as potassium hydroxide in the sodium hydroxide solution.

Sulfur is required at about 0.8 percent based on the weight of the cells or 0.0089 grams per gram of cells. The remaining nutrients are calcium chloride 0.01 gm per gram of cells, magnesium sulfate 0.01 gm, yeast lysate 0.03 grams per gram of cells, manganese sulfate 0.001, cobaltous chloride 0.001, zinc sulfate 0.001, trace minerals 0.01 ml per gram and antifoam agent P-2000 at 0.001 ml per gram.

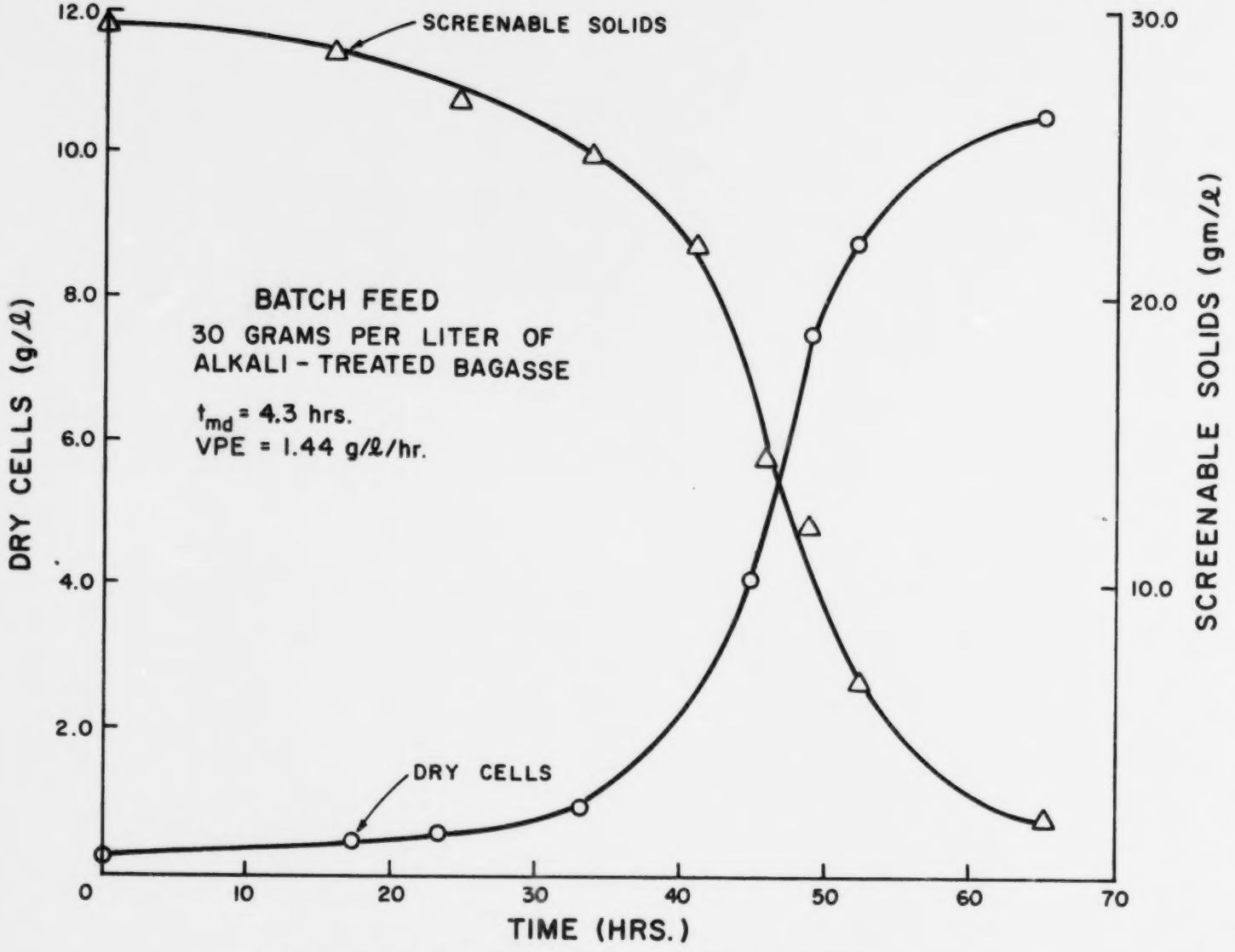
Fermentation

The productivity of a fermentation process is based on the volumetric production efficiency (VPE) as previously mentioned. The history of this project has been such that economically feasible VPE's have been extremely difficult to achieve because of the very low cell concentrations obtainable in the fermenters. Very recently, however, this situation has shown a remarkable improvement. Cell concentrations have been obtained in the large fermenter of up to 6 grams per liter using intermittant feed. These data are shown in Figure 2. Cell concentrations of 10 grams per liter have been obtained on the 14 liter fermenter with growth all in the log phase. These data are shown in Figure 3. It is recognized that data on the 14 liter fermenter is not the same as continuous operating data on the 535 liter fermenter. However, the investigators at the pilot plant have been extremely successful in the past in reproducing batch data on the continuous unit.



GROWTH IN 535ℓ CONTINOUS FERMENTER
RUN 535-38 6/19/71

FIGURE 2



GROWTH IN 14 LITER FERMENTER
RUN 14-2-14 6/28/71 FIGURE 3

The batch data can be converted to a continuous basis using the method of Adams and Hungate (42). These calculations show mass doubling times of 4.3 hours and cell concentrations of 10.0 grams per liter in log phase of growth. The product of the cell concentration and the retention time gives 1.44 grams per liter per hour for VPE. This means that if the fermenter could be run completely full that 0.695 liters of fermenter volume would be required to produce 1 gm per hour of product. However, some free board is necessary for foaming and some liquid expansion occurs due to aeration of the liquid. It should be safe to allow for approximately a 45% increase in volume to take care of these factors. Based on this increase, about 1 liter of fermenter volume would be required to produce 1 gram per hour of product.

Aeration Costs

The continuous fermenter being used at the pilot plant is a turbine type which appears to give efficient oxygen transfer. The internal construction consists of 2 doughnut rings with one turbine blade above and below each ring. The rising air is swept into the turbines while the descending liquid comes down around the outer periphery of the rings. Good growth rates are maintained at about 0.4 liters of air per liter of liquid volume per minute. Any commercial unit would probably operate as well, even one with a draft tube air lift arrangement, because of the small oxygen requirements. The amount of oxygen required is slightly more than 1 gram per gram of cells. Since only 0.016 grams of cells per liter per minute are to be produced and 0.4 liters of air has 0.138 grams of oxygen this requires only slightly over 12% efficiency for

the mass transfer. This is within the range of commercial fermenter operations where about 15% efficiency of oxygen transfer is normal.

The compression costs for the air will be dependent upon the method of sterilization of the air stream, the pressure drop across the spargers, the liquid head height in the fermenter, and the pressure drop in the lines. One way to obtain sterilization of the air stream is to pump the air to a high pressure in an adiabatic compressor. The resulting temperature increase will then cause sterilization. The residence time at this elevated temperature will be brief and the relative humidity will be far below saturation which may require a much higher temperature then would normally be expected. From thermodynamic considerations, the ideal work for all adiabatic compressions is given by:

$$W_s = \frac{\gamma RT}{\gamma - 1}\left[\left(\frac{P_2}{P_1}\right)^{\frac{\gamma-1}{\gamma}} - 1\right]$$

where γ is equal to the ratio of the heat capacity at constant pressure to the heat capacity of constant volume, which for air is 1.395.

Assuming that the initial temperature of the air is 540° Rankine and that the final pressure is to be 115 psia. The work required would be 2990 BTU per pound mole of air. The work load can also be calculated by:

$$P_{r_2} = P_{r_1}\left(\frac{P_2}{P_1}\right)$$

from Hougan, Watson and Ragatz, Volume II, pg. 668 which comes out to 2991 BTU per pound mole. The final temperature will be 965°R or 505°F. Because of the short residence time and low relative humidity, this high temperature may be required to obtain complete sterilization.

Another way to obtain sterilization of the air stream is to pass the air at high velocity through very fine glass wool. With this method, the encroaching organisms are impinged on the small glass fibers and sterilization

is obtained. Since only about 10 to 15 pounds pressure are dropped through these filters, and their initial cost is nominal, it would probably be cheaper to follow this route.

The total pressure drop then could be calculated as follows:

5 PSI due to pipe resistance

15 PSI through fiber glass filter

10 PSI to overcome liquid head in the fermenter

10 PSI to be dropped across the spargers

Total = 40 psig or 55 psia.

$$W_g = \frac{-(1.395)(1.98(540)}{1.395-1.0}\left[\left(\frac{55}{14.7}\right)^{\frac{.395}{1.395}} - 1\right] = 1710 \text{ Btu/lb-mole}$$

Since 0.4 liters of air per liter of liquid volume per minute are to be pumped, this requires 0.00159 HP per liter of liquid volume for the compression or 0.00119 Kilowatts. Power charges are usually something less than a penny a Kilowatt hour so compressor costs will be about 0.00119 cents per liter of fermenter capacity per hour.

Cell Harvest

A continuous sludge discharge centrifuge which is normally used for the harvest of yeast cells was not considered practical here because of the very small size of bacterial cells. Several alternative methods of cell harvest have, therefore, been looked at.

Hyperfiltration

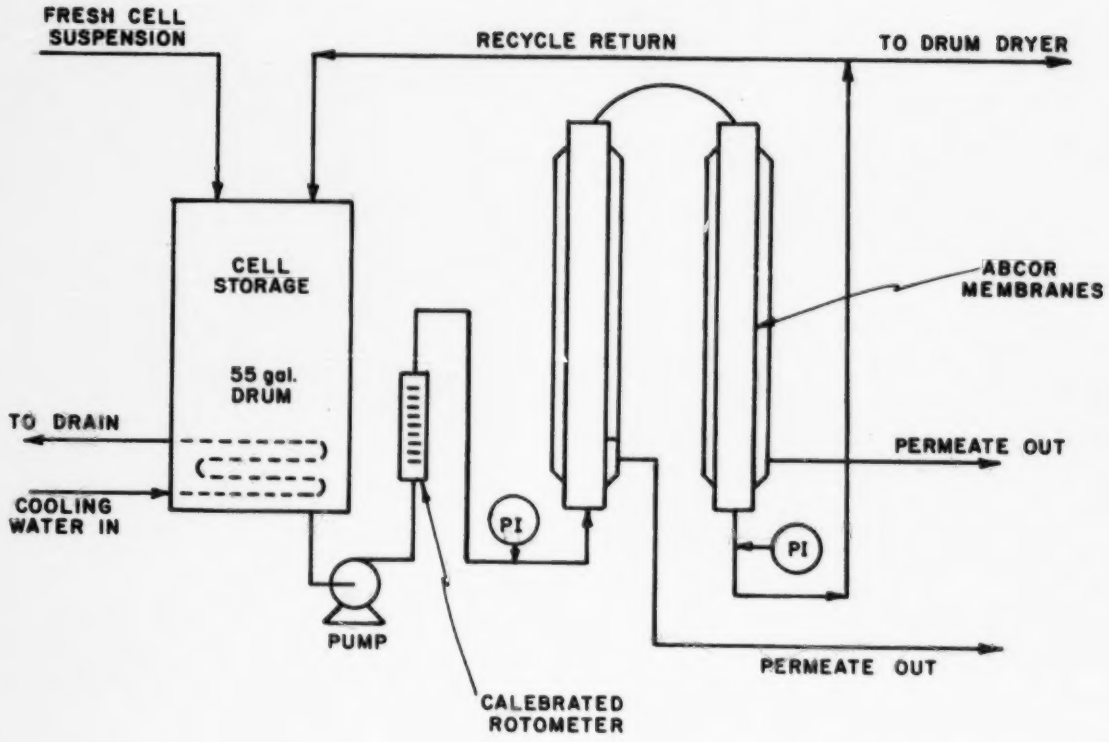
One of the most promising of these has been hyperfiltration. Wang, Sinskey and Sonoyama (43) did a series of tests using Abcor's cellulose acetate HF-200 membrane to test its efficiency in concentrating and purifying a crude enzyme

(trypsin) preparation. They reported high flux rates for the permeate initially but these rates gradually dropped off with continued operation due to a build up of macromolecules on the surface of the membrane. The retentate they worked with had a molecular weight of 23,800. They also found that the presence of soluble ions such as salt, ammonium sulfate, etc. gave a sharp reduction in permeate rate up to 0.05 moles per liter and this reduction gradually leveled off at higher concentrations.

A study was undertaken in this work to determine if hyperfiltration was practical for concentrating microbial cells. According to the basic theory of reverse osmosis, the driving force for passage of the permeate through the membrane is proportional to the difference in osmotic pressure between the liquids on the 2 sides. In order to counterbalance the lower partial pressure of water on the inside of the membrane, an additional pressure must be applied. The amount of pressure required is proportional to the number of particles present but not necessarily to their size.

In other words, the larger the size of the particles in the solution to be separated the lower the total pressure necessary to obtain separation at a given rate. In order to obtain high flux rates with sea water, pressures on the order of 1000 pounds are required. For protein separation because of their higher molecular weight, pressures of 50 to 75 psi are required. Microbial cells are very high in molecular weight compared to protein molecules and therefore would not be expected to need such a large driving force.

For our experimental work two Abcor membranes were obtained, each was one inch in diameter and sufficiently long to make 1.1 square feet of membrane area. A schematic diagram of the assembly is shown in Figure 4. Because of difficulty that other investigators have had with cake build-up on the membrane surfaces, a high-through-put pump with low head pressure was installed and the two membranes hooked in series. High flux rates were noticed at the beginning of each run



EXPERIMENTAL
HYPERFILTRATION UNIT

FIGURE 4

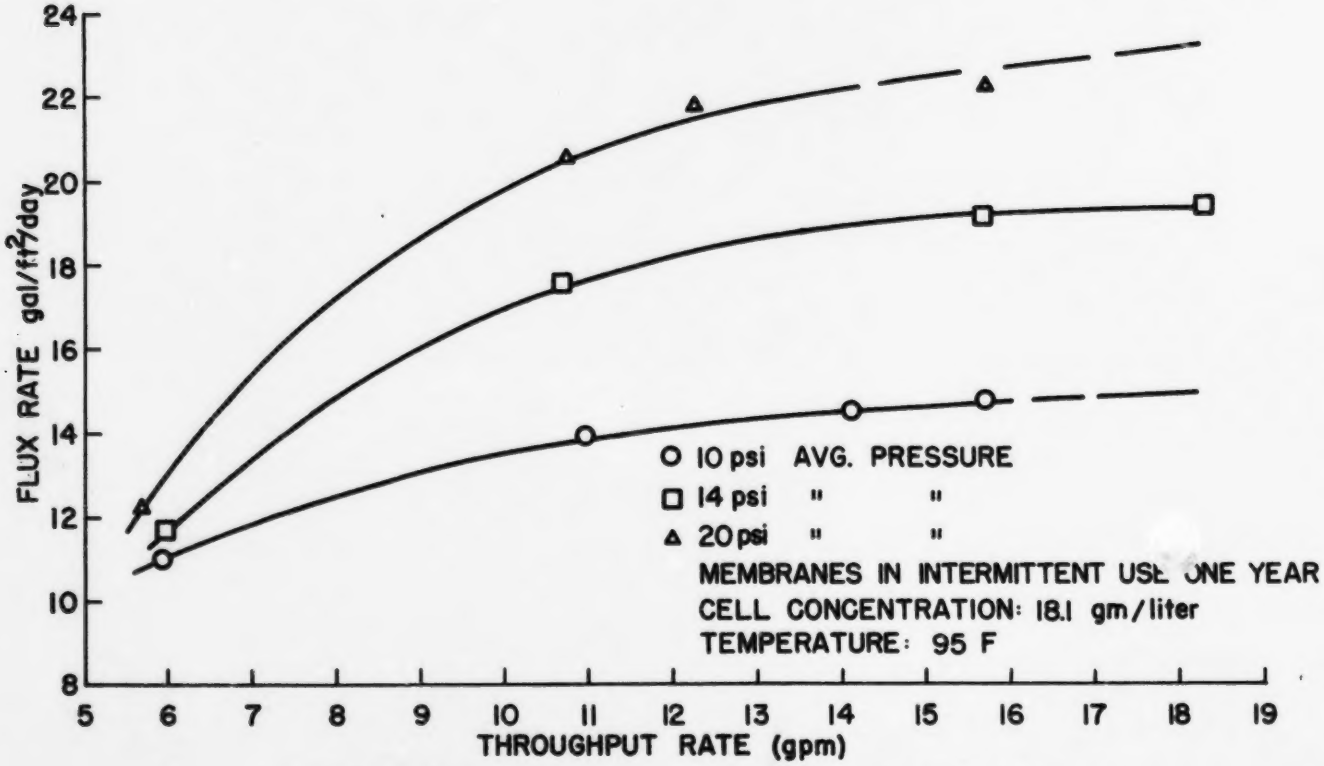
but these rates would level off after about 30 minutes of continuous operation and very little further drop in flux rates was observed from then on.

The linear velocity through the tubes was maintained at from 1.8 to 7.0 linear feet per second and the head pressure was varied from 10 to 30 psig. Flux rates on fresh membranes ran up to nearly 30 gallons per foot square per day and this seemed to fall off only slightly with a 20 fold increase in cell concentration. After the membranes had been used intermittantly for 6 months the flux rates had dropped somewhat. When flow rates became too low, the membranes were soaked in a cold water detergent which contained a proteolytic enzyme. This treatment would usually reestablish higher flux rates which would remain that way until taken out of service for lack of cells to feed.

Figure 5 shows that the permeate rates increase quite sharply with increases in linear velocity or turbulence up to about 290 linear feet per minute. Above this velocity, further increases had very little effect on permeate rate. This corresponds to a Reynolds number of about 40,000 for the cut off point. Michaels (44) and Rickles and Friedlander (45) have described a phenomenon known as, "concentration polarization" which is demonstrated by a decrease in permeate rate with time, partially due to a cake build-up on the membrane surface and partly due to entanglement of macromolecules in the pores of the membrane passageways.

The cake thickness can reportedly be controlled by the Reynolds number. These data seem to indicate that perhaps this is true, up to a Reynolds number of about 40,000 for this particular system. Figures 6 and 7 show the difference in permeate rates for membranes HFA 200 and 300. Obviously HFA 300 would be the choice for this separation.

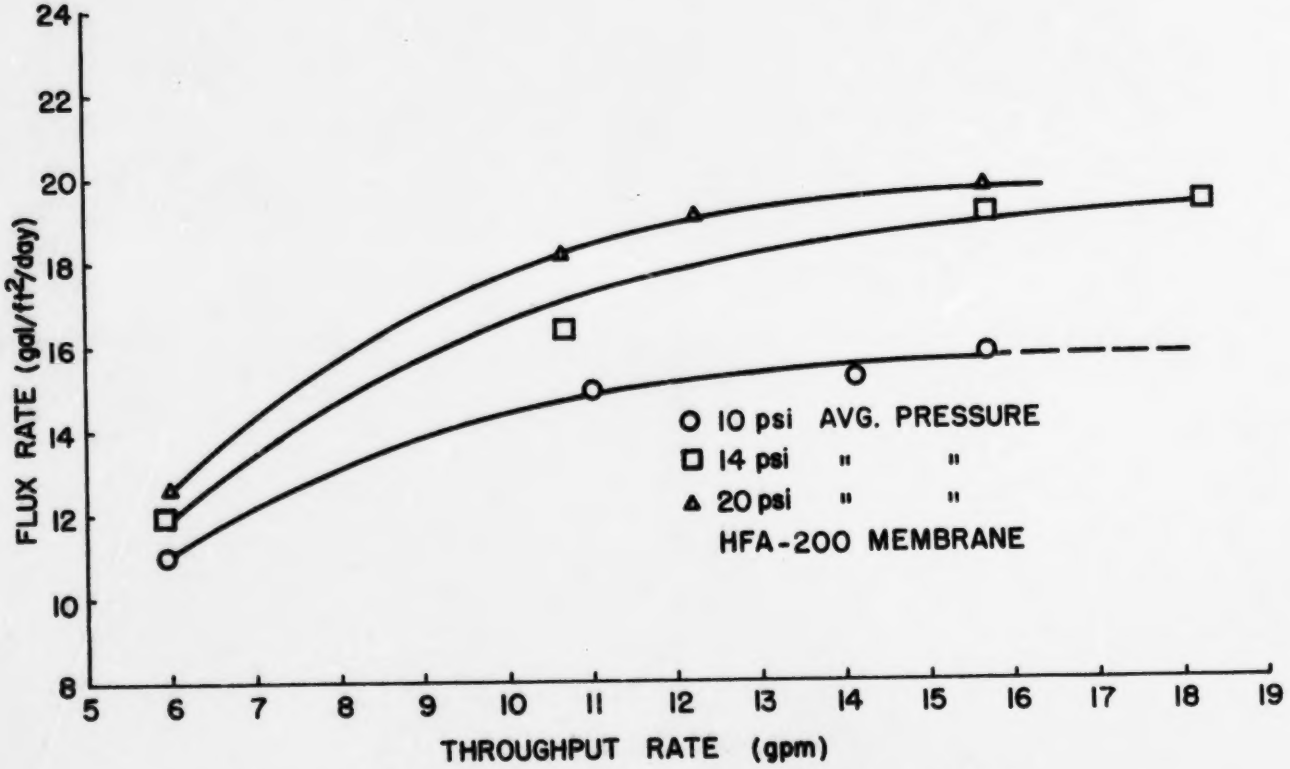
As noted earlier, microbial cells are large compared to enzymes and the source of macromolecules that might plug the pores had been a matter of some



PERMEATE RATE vs THROUGHPUT RATE

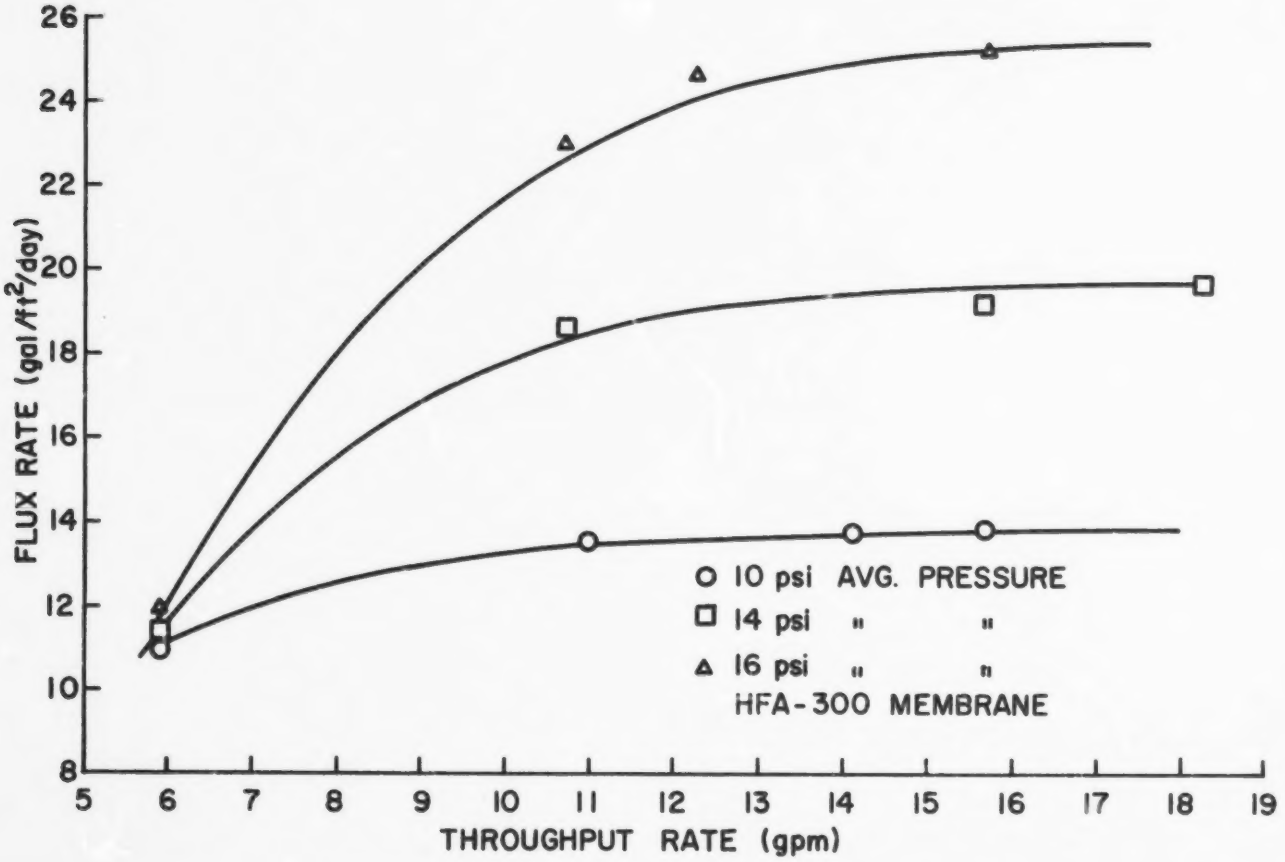
AVERAGE OF gal/ft²/day FOR HFA-200 & HFA-300

FIGURE 5



PERMEATE RATE vs. THROUGHPUT RATE

FIGURE 6



PERMEATE RATE vs THROUGPUT RATE

FIGURE 7

interest. It is true that the menstrum from the fermenter must contain enzymes, however, the cellulase enzymes are supposedly not extracellular. Other enzymes may be present but these are not expected to be extremely high in molecular weight. Certainly some cells lyse during filtration releasing protein and cell debris which could contribute to the source of macromolecules but this source should also be minimal.

Measurements were made to see if nucleic acids were being released by the cells due to autohydrolysis of the nucleic acid from the thermal shock put in by the high input power to the pump. It was found that nucleic acids were being released by the cells and apparently diffusing to the surfaces and the severe turbulence created by the pump head was washing them off into the liquid so that they could then diffuse out through the membrane walls. Although very little time was spent studying this phenomena, it does appear that nucleic acids may be partially removed by autohydrolysis and diffusion while at the same time the cell concentration is being increased. This may turn out to be an essential step if the product is to be used for human food.

Permeate rates of 24 gallons per foot square per day were observed with the HFA 300 membrane at an average pressure of 20 psi. Each set of membranes and fittings dropped 6.5 psi at 12 gallons per minute flow rate. For design purposes this data could be used without too much risk. Assuming that the feed stream entered the membranes at 10 grams of cells per liter or 1 percent and left at 5 weight percent, this means that 80 percent of the water must be removed before leaving. This requires that 0.22 square feet of membrane will be needed for each liter of fermenter liquid volume to produce 10 grams per hour of cells or 0.022 square feet per gram of cells per hour.

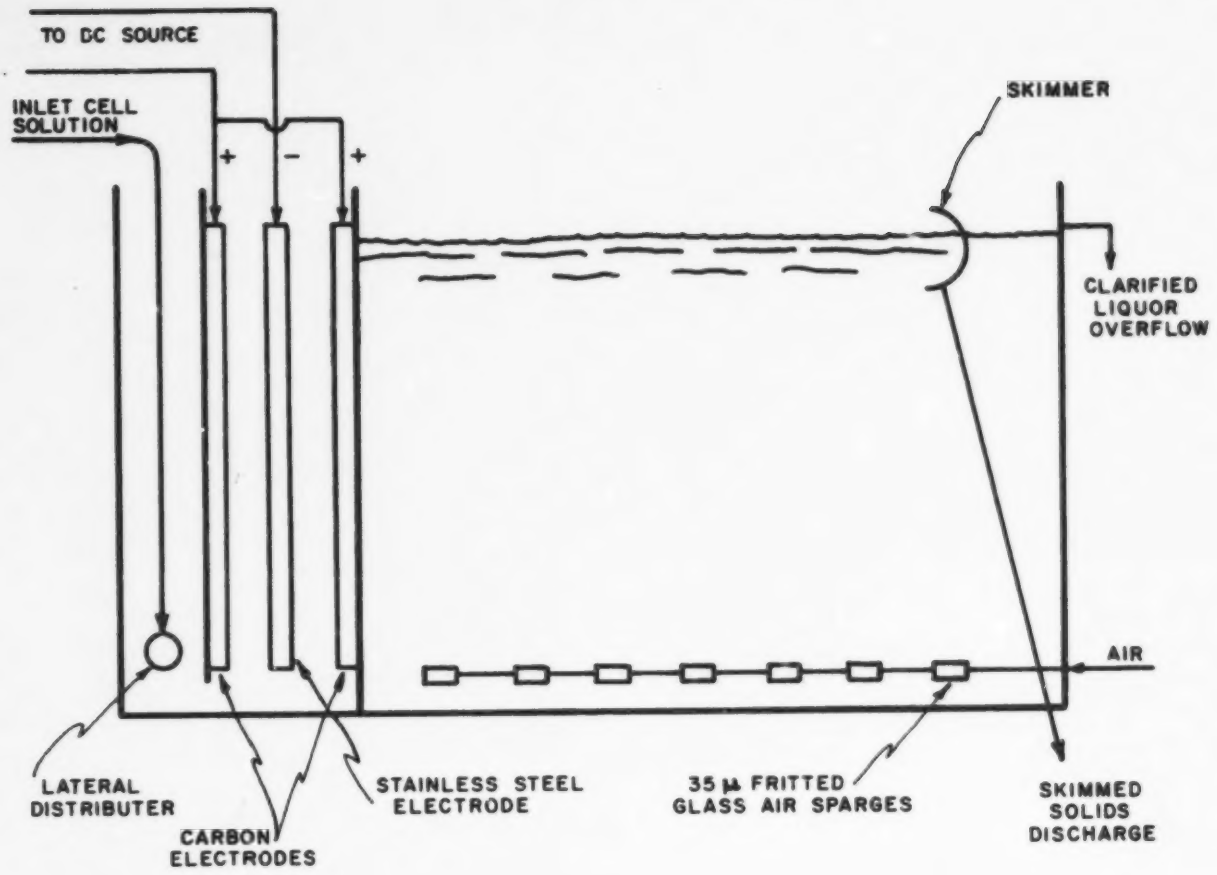
Since the pump must put out sufficient pressure to maintain an average driving force throughout the membrane pass of 20 psi, the inlet feed pressure

will have to be 40 psi. The brake horsepower required for this operation is then 0.35 HP per membrane. At 90% efficiency of power conversion this amounts to 0.29 Kilowatt-hours per hour or approximately 0.29 cents for electrical costs to operate one membrane to obtain the removal of 900 ml of liquid. In an actual installation it might be appropriate to consider membranes that are smaller in diameter so that the liquid volume would not have to be so high to obtain the necessary turbulence.

Electrolytic Flotation

A simple test unit was constructed with two carbon electrodes separated by approximately 1.5 inches from a stainless steel electrode in the center of a sandwich construction. A rectifier was used to convert the alternating current to direct current. The voltage was adjustable as was the distance between the electrodes. The cell solution was allowed to pass slowly up between the plates where electrolytic action took place. Then about 6 amperes was allowed to flow at 12 volts, flocculation of the suspended solids occurred. Because of the presence of small gas bubbles on the surfaces of the coagulated solids they floated to the surface. A flotation chamber was built into the unit to hold the suspended solids at the liquid surface so they could be removed with a skimmer. A diagram of the unit is shown in Figure 8.

The test results showed that when 12 volts and 8 amps were used at a flow rate of 1.5 gallons per hour of solution, essentially complete flotation did occur. The floated product not only included all of the cells but essentially all of the lignin. The solids content of the foam was about 5 weight percent. More skill and practice with the unit may have resulted in an increase in this value.



ELECTROLYTIC FLOTATION UNIT

FIGURE 8

The foams collected at the cathode and anode were completely different in appearance. That at the cathode was white and formed a stable foam, while that at the two annodes was dark brown in color. The pH of the white foam from the cathode was approximately 11.0 while that at the annode was about 3.0. The pH of the clarified liquor was usually at seven. The formation of the dark colored material at the anode would stop after the solution had been completely clarified. Some speculation was made that the white material could have been the sodium salt of the proteins and that the brown material was lignin. However, this has not been confirmed because of the difficulty in separating the two streams as the unit was originally designed.

The economics of this approach are somewhat difficult to evaluate because of its sensitivity to flow rate. No attempt was made to optimize the flow in the limited time available for the experiments. If one assumes a flow rate of 1.5 gallons per hour and a power of 72 watts to effect clarification, this means that 13 watts per liter per hour will be consumed. At a penny a kilowatt hour then 0.022 cents per liter per hour will be the electricity costs for this operation based on 60% power efficiency of the rectifier. This operation would increase the cell concentration from 1 percent to 5 percent.

Flocculation

Although flocculation is the current method of cell harvest used in the pilot plant to obtain test cells, very little time has been spent on working out the best technique and the minimum amount of flocculent to use. Good results have been obtained using polyethylenimine (PEI) at about 0.01 grams per gram of cells to bring the concentration of the underflow to about 5 weight percent. Using these figures with PEI about one dollar a pound, this would give a cost of 1.0 cent per pound of cells or 0.0022 cents per gram of cells.

This may be somewhat too high to be practical.

Other investigators have been able to obtain flocculation with roughly one tenth these quantities using the proper flocculent and proper equipment. Earlier in this report it was pointed out that McGregor and Finn (25) obtained a 10 fold improvement in the effectiveness of a flocculent if the cells were first subjected to violent agitation to wash off the RNA and proteins that had leaked to the cell surface. Our own work on hyperfiltration showed that such violent agitation was obtained inside a centrifugal pump. It would appear from this that the best place to add the flocculent would be through a small inlet into the casing of a centrifugal pump. This idea will be tried as soon as time and money permit detailed studies on cell harvest.

Air Flotation

Some limited experiments have also been run using air flotation. This system seems to show the most promise, but insufficient data on our own system has been obtained to be precise on the economic analysis. Jones (46) has made a careful study on the removal of suspended organism by air flotation and his data should reasonably well represent what might be expected on our own system. His data shows that with the combined use of dissolved air and cationic flocculents, a much better solution can be obtained then with either alone.

Applying his data to our system may be slightly risky, however, if one chooses the most stringent operating conditions that he has found necessary much of the risk will have been removed. At a feed rate of 165 gallons per minute to a 250 square foot separator he was able to decrease the solids content from 11.9 g/l in the feed to 0.035 g/l in the effluent. This was at a loading of 982 pounds of solids per hour or 3.9 pounds of solids per

hour per square foot of surface area. He used an air to solids ratio of 0.02 lb per lb at 60 psia air pressure and 0.05 lb of cationic polyelectrolyte per ton of dry solids. The collected solids contained 95% liquid. The solids removal was 99.7%

Transposing these data to our own system being certain to build in a safety factor one obtains the following information. The air required for this floatation would be 1.0×10^{-5} gm of air per gm of solids at 60 psia but this is such a negligible amount that it can be included in the compressor costs for air to the fermenters. Under the very worst conditions, the amount of flocculent required is 0.0025 gm per gm of cells. At $1.00 per pound this is 0.25 cents per pound of cells or $\underline{5.52 \times 10^{-4}}$ $\underline{\text{cents per gm of cells}}$. This cost data will be used in the economic analysis of the process.

Drum Drying

The final drying of the cells will be done on an atmospheric drum dryer. Although this may not be the cheapest method of drying, the final high temperature attained by the product renders the cells non-viable. Furthermore, feeding studies have shown that this product is somewhat more digestible than products obtained by other techniques. The drum dryer now in use operates on 80 psig steam and about 2.5 pounds of product per hour per square feet of surface is obtained. The capacity of the dryer appears to be somewhat dependent on the degree of fit of the doctor blade or on how thoroughly the dried cells are scraped off by each pass under the blade.

The amount of steam required will depend on the liquid content of the cells coming to the dryer and this in turn will depend on the method used for concentrating the cells. Two of the methods investigated have given cell concentrations of 5%. It is almost a certainty that given sufficient

time to work on these steps that the cell concentrations can be increased, however, the 5% figure will be used in this analysis to be absolutely certain of the cost data.

The product after drying normally contains about 5% moisture so based on 100 lbs of feed at 5% solids, 95 lbs of water must be vaporized. The latent heat of vaporization of water at atmospheric pressure is 970 Btu per pound while the heat available from 80 psig steam is nearly the same. That means that 100 pounds of steam will be required to vaporize the water from 5 lb of product. The normal cost of 80 psig steam is 60 cents per 1000 lbs so that steam costs will run 1.20 cents per pound of product.

The product leaving the dryer will go either to a granulating unit or a hammer mill depending on the final use. For most applications a granulator would be desirable because this would increase the bulk density of the product and make the cells into sufficiently large particles that they could be easily handled without being blown around by local air currents.

Waste Stream Composition

The effluent stream leaving the cell separator will contain essentially all of the lignin that entered the processing plant. This means that for every gram of feed to the plant 0.1 gram of lignin will be discharged or approximately 0.263 grams for every gram of product produced. As mentioned earlier, it may be possible to recover the lignin either by acidification followed by flotation and skimming or by electrolytic flotation. Whether or not this would be a practical step would depend on the value of the product, or if the discharge of lignin to the waterways were particularly undersirable then this could have a strong bearing on the installation of recovery techniques.

Since the feed stream has a relatively short residence time in the fermenter, complete equilibrium is not reached between the organism and the soluble carbohydrates.

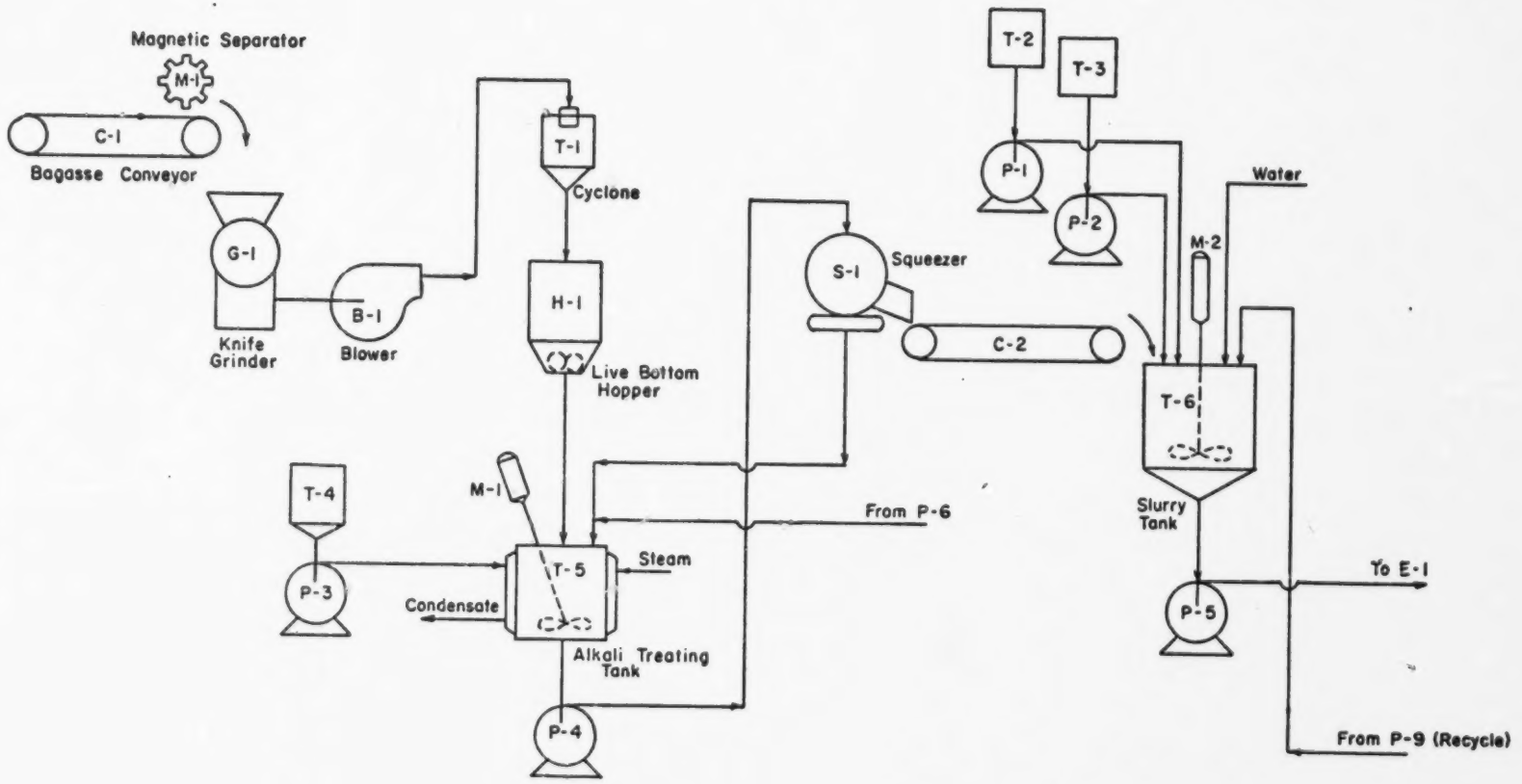
Generally about 400 milligrams per liter or 400 parts per million of soluble sugars will be present in the effluent stream, this means that ponding of the effluent will be required to maintain acceptable BOD standards. This would generally require only a short retention time because some cells will be present to hasten consumption of the discharged components.

Only 90% utilization of the nutrients has been obtained so far at the pilot plant, so that if no recycle is done a total of 0.0172 grams of N, P, and K will be discharged per gram of product produced.

Stakhorskaya and Giterman (47) have shown that in yeast production partial recycle can be used to cut the effluent nutrients essentially in half. This also cuts in half the amount of fresh water required for the process. No attempts at recycle have yet been made in the pilot plant although the idea is facinating in terms of cellulolytic enzymes. If indeed, any of these enzymes could be returned and combined with the feed stream, after it had been sterilized so that the enzymes would not be denatured, the production rates should be greatly increased. Such a process step would entail sterile cell separation techniques if fermentation is to remain sterile. This concept will probably not be tested for some time due to the press of more immediate problems.

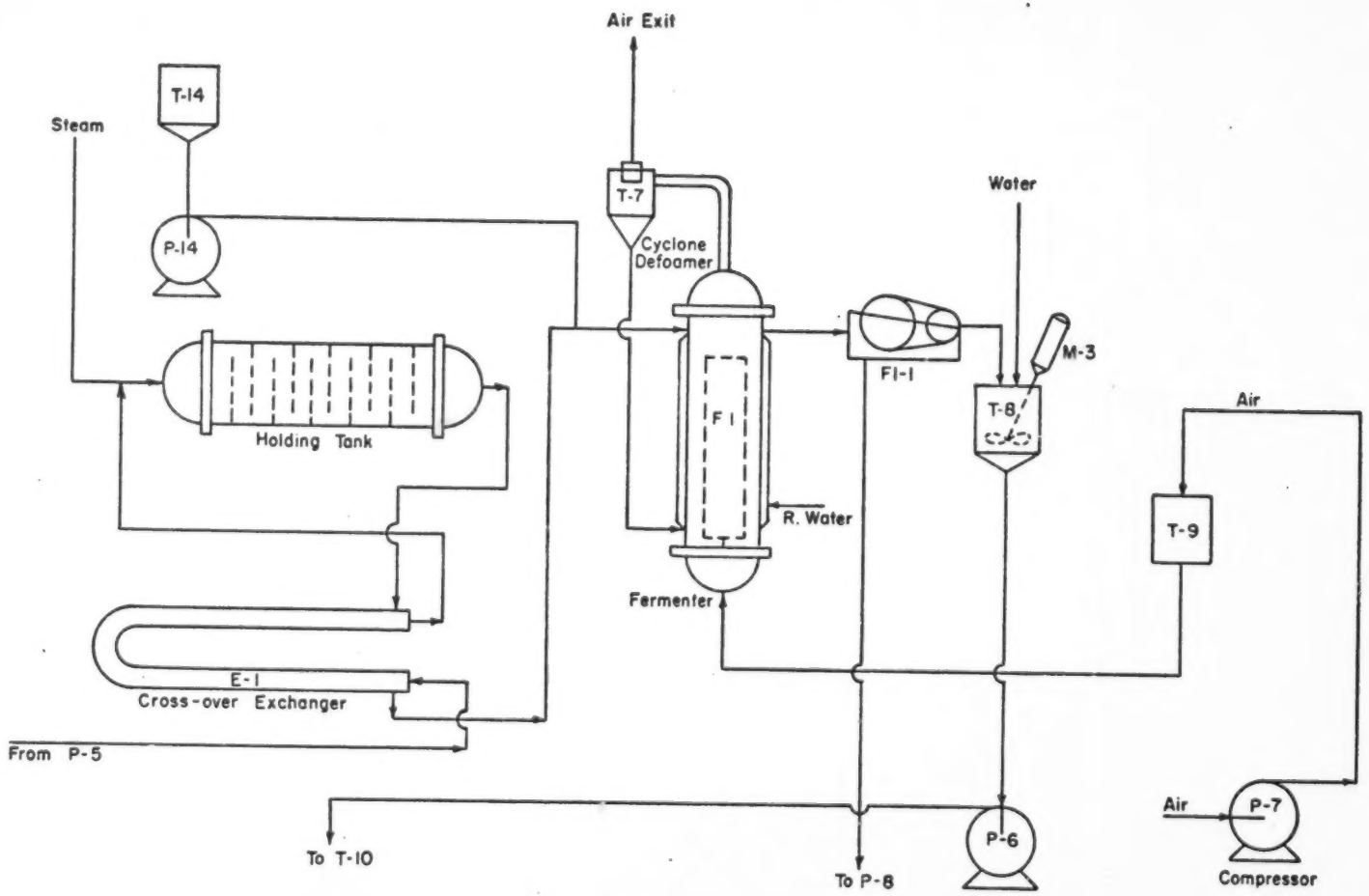
Economic Analysis

A schematic flow sheet of the process is shown in Figures 9, 10, 11. The process equipment is tabulated in Table VIII. Meller (22) in his report to the Bureau of Solid Waste did an economic study on the production of yeast from cellulose. He based the production cost for this process on a plant to produce 1540 lbs per hour of yeast cells using 80 tons per day of cellulose feed for case 1 and for 500 tons per day of cellulose feed for case 2. Since the objective



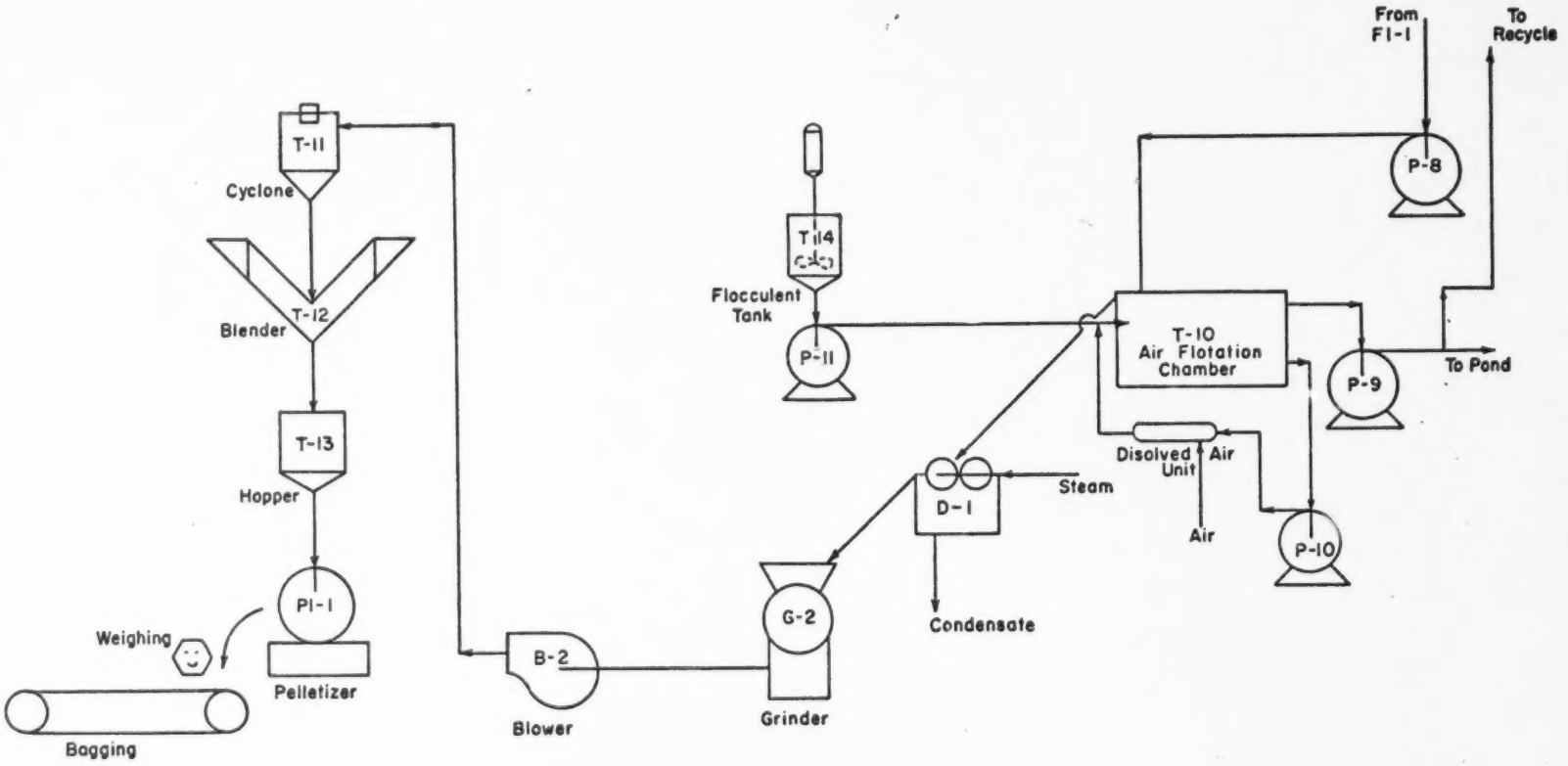
SOLIDS GRINDING AND ALKALI TREATMENT

FIGURE 9



STERILIZATION AND FERMENTATION

FIGURE 10



CELL HARVEST AND PRODUCT BAGGING

FIGURE 11

TABLE VIII

Equipment List
ENR for 1972 - 1270

Flow Sheet Number	Number of Items	Description	Size	Installed Equipment Cost
Mg-1	2	magnetic separator	18"	10,000
C-1	2	bagasse conveyors	20 ft.	9,800
G-1	2	bagasse grinders	100 HP	31,200
B-1	2	blowers (included in grinder costs)		
T-1	1	cyclone	900CFM	4,200
H-1	1	live bottom hopper	40 ft^3	14,000
T-5	1	alkali treating (monel) tank	10,000 gal.	35,000
M-1	1	motor for alkali tank	40HP	7,500
S-1	1	alkali squeezer motor	80HP	48,000
C-2	1	conveyor (rubber)	20 ft.	4,900
T-6	1	slurry tank (S.S.)	1000 gal.	4,100
M-2	1	slurry tank mixer	5HP	500
E-1	1	cross over exchanger	725 ft^2	19,000
T-15	1	sterilization holding tank	500 gal.	15,200
T-7	1	cyclone defoamer		2,000
F-1	1	jacketed fermenter (425,000 liter)		105,000
FL-1	1	continuous solid bowl centrifugal	30" bowl	38,000
T-8	1	cellulose reslurry tank	100 gal.	2,000
T-9	1	glass wool air filter		800
T-10	1	dissolved air floatation	300 sq. ft.	45,000
D-1	1	drum dryer	615 ft^2	76,000

Table VIII (Cont'd)

Flow Sheet Number	Number of Items	Description	Size	Installed Equipment Cost
T-14	1	flocculent make-up tank	50 gal.	750
G-2	1	product granulator	40HP	24,000
B-2	1	blower		1,000
T-11	1	cyclone		800
T-12	1	rotary blender	50 ft^2	6,500
T-13	1	product hopper	1000 ft^3	2,000
A-1	1	weighing & bagging	2 bgs/min.	5,000
T-14	1	Hcl tank (fiberglass)	1,000 gal.	2,000
P-7	1	rotary compressor	700HP (6,250 cu ft/min) 60 psi	27,000
				$541,250
PUMPS				
P-1	1	Fertilizer pump	20 GPM (metering)	1,000
P-2	1	trace minerals, anti-foam	5.GPM (metering)	600
P-3	1	yeast lysate, fertilizers	50 GPM (metering)	1,000
P-14	1	pump (pH controlled)	1.3 GPM	500
P-4	1	alkali (centrifugal)	13.2 GPM	950
P-5	1	slurry (centrifugal)	333 GPM	2,000
P-6	1	slurry recycle	17.7 GPM	1,000
P-9	1	effluent discharge	300 GPM	1,950
P-10	1	included with unit		-
P-11	1	included with unit		-
				$550,250

of both these processes is the same, the economic analysis here will be made on that same basis for easy comparison. The same basis is meant to mean the same amount of product per hour, not necessarily the same amount of feed. Perhaps an even better comparison would be the same amount of protein produced per hour. The bacteria produced in this research are being analyzed by amino acid analysis to be ~ 55% or more protein compared to 51% protein in yeast. Protein is the usual basis for comparison of SCP production plants.

Equipment Sizing

A material balance must be made around the entire process so that allowance can be made for any losses before the feed can be determined if the design basis is specified by the amount of product. Since some losses can occur by incomplete cell recovery, cyclone vents, pump leaks, etc. an assumption of 98% recovery should be on the safe side. At 0.38 lbs of cells per pound of feed, this gives 4140 lbs per hour of bagasse feed.

A stream factor of 0.9 is assigned to the equipment sizes to allow for downtime, turnabout, strikes, etc. The equipment sizes will be increased by 10% to allow for this. However, this factor does not apply to utilities and raw materials consumption.

One knife grinder can grind over 4600 pounds per hour with a 100 horsepower motor. Because they must occasionally be taken out of service and the knives ground, a spare should be installed. The cost of this will be taken from already purchased equipment and scaled up. The cost of other equipment has been obtained from (48,49,50,51,52) using a projected ENR from Dryden and Furlow (48) for the year 1972. The reason for projecting the cost to next year is because it would be physically impossible to use the data presented in this report to purchase equipment any sooner than that. This would make the data not directly

comparable to Meller's data on the production of yeast from cellulose but it is more realistic.

Table IX lists the raw materials that will be consumed to produce 1540 pounds per hour of product. This cost includes a charge not only for the waste cellulose feed but also a charge for every possible nutrient the microorganisms require. These data, then represent the maximum costs for raw materials and future modifications could markedly decrease these numbers. For example, the yeast lysate is used to provide a growth factor, preliminary experiments have shown that chicken manure will do equally as well. The caustic being used is 4.65 cents per pound, it could easily be replaced with cell effluent, a very cheap mixture of salt and caustic from diaphram cells. If the brine being used for the electrolysis were sea water, additional trace elements would be available for the microorganisms.

The equipment listed in Table VIII has with it the sizes and the installed costs for the year 1972. The equipment and numbers correspond to those listed in the flow sheets of Figures 9, 10 and 11. The equipment cost data have been used to prepare an estimate of the required capital to build a plant to produce 1540 pounds per hour (500 tons per month) of SCP from waste cellulose. These data are shown in Table X.

Based on equipment costs, construction costs, raw material costs, return on investment, etc., a manufacturing cost estimate is shown in Table XI for a plant to produce 500 tons per month of single cell protein from cellulosic waste. The production cost per pound of this product is 12.90 cents for this rate of production. The cost found by Meller (22) is over 15 cents per pound for yeast grown on cellulose at the same hourly rate but without a return on investment.

Table IX

Raw Materials for 500 tons per Month
of SCP from Waste Cellulose

Raw Material	#/hr		cent/#		$ Cost per hour
Bagasse	4,400		0.25		11.00
NaOH (73%)	200		4.65		9.30
Hcl (16°Bé)	182		1.3		2.36
Ammonia (NH_3)	(217)	$(\frac{17}{14})$	(3.0)	=	7.90
53% H_3PO_4 rock(feed grade) P.0163	(27.2)	$(\frac{98}{31})$	(3.1)		2.66
Kcl (99.5%) K.0256	(43.0)	$(\frac{74.5}{39})$	(1.65)		1.35
$MgSO_4$	(45.0)		(3.1)		1.40
$CaCl_2$	(45.0)		(2.2)		1.00
Trace Minerals (sea salt)	(30)		(2.0)		.60
Antifoam (polyglcol 2000)	(15)		(40)		6.00
Yeast Lysate	(15)		(10)		1.50
1540 lbs/hr.					45.07 = 2.93¢/#

Table X

Estimate of Required Capital for
a 500 tons per month SCP Plant
(1540#/hr of product 73% Protein)
projected for 1972

1.	Purchased equipment	550,250
2.	Installation including control & instrumentation	330,000
	total purchased equipment	880,250
	piping, electrical (20% of PE)	176,500
	Building & services (40% of PE)	352,000
	yard improvement (10% of PE)	88,000
	service facilities (50% of PE)	440,125
	Land (5% of PE)	44,012
	Engineering & supervision (13% of PE)	115,000
	Construction expense (14% of PE)	123,000
	Contractors fee (3% of PE)	26,400
	Contingincy (12.3% of PE)	108,000
	Fixed capital	2,353,287
	Working capital	122,000
		2,475,287

Table XI

Manufacturing Cost Estimate
500 tons per month of SCP from Waste Cellulose

A. Direct Production Cost	Unit/hr	cost cents/unit	cost dollars/hr.
Raw Material			
Bagasse	4,400	0.25	11.00
NaOH (3%)	200	4.65	9.30
	182	1.30	2.36
Ammonia	217	3.0	7.90
Phosphoric acid (53%)	27	3.1	2.66
Potassium chloride	43	1.65	1.35
Magnesium sulphate	45	3.1	1.40
Calcium chloride	45	2.2	1.00
Trace minerals (sea salt)	30	2.0	.60
Anti-foam (P-2000)	15	40	6.00
Yeast lysate	15	10	1.50
			45.07
Utilities			
Steam 40M lb/hr at 60¢/M			24.00
Electricity 745 KW/hr/hr at 0.8/KW hr.			6.00
Potable water 12M Gal/hr at 20¢/M			2.40
Operating labor 2 men/shift at $3.00/hr			6.00
Supervision - 1 man for 4 hrs. (6.00/hr)			1.00
Fringe benefits & operating supplies (25% of labor)			1.75
Maintenance (7% of fixed capital)			18.80
Direct Production Cost			59.95
B. Fixed Charges			
1. Depreciation - 10 year straight line(salvage value 10% of initial cost)			24.2
2. Insurance (1% of capital)			2.62
3. Local taxes (2% of capital)			5.24
C. Plant Overhead			
1. Medical, payroll, packaging, control, etc. (60% of labor, including maintenance)			11.90

Table XI (Cont'd)

	cost dollars/hr
D. General Expenses	
1. Administrative (15% of labor plus 13% of maintenance)	3.50
2. Interest (6% of Fixed capital)	16.10
3. Profit (10% return on Investment)	29.30
	92.86
Total	$197.88/hr.
Product	1540.00 lb/hr.
Price = 12.90 cents/#	

Even this comparison does not tell the whole story because Meller's costs were based on 1968 prices while these are based on 1972 prices. Capital costs will have increased by over 18% according to Dryden and Furlow (48) over that 4 year period. The correct way to compare single cell protein products are on a basis of pure protein. By this technique the bacteria product would be about 23 cents per pound of pure portein while yeast from the same raw material would be 29 cents per pound of protein; considerably larger. Table XII shows how the production cost decreases with increases in the rate of production.

Table XII

Hourly Manufacturing Costs
for Various Size Plants

Item	500 tons per month	1,000 tons per month	2,000 tons per month	3,000 tons per month	6,000 tons per month
Fixed Capital Investment	2,353,000	3,560,000	5,400,000	6,900,000	10,420,000
Raw materials	45.07	90.14	180.28	270.42	540.84
Utilities	32.40	64.80	129.60	194.40	388.80
Operating labor	6.0	6.0	6.0	6.0	6.0
Supervisor	1.0	1.0	1.0	1.0	1.0
Fringe benefits & operating supplies	1.70	1.78	1.70	1.70	1.70
Maintenance & Repairs	18.80	28.50	43.20	55.00	83.40
Depreciation	24.20	36.60	55.40	70.80	107.10
Local Taxes	5.20	8.10	12.30	15.80	23.80
Insurance	2.70	4.10	6.10	7.90	11.90
Overhead	11.90	15.90	21.90	26.80	38.40
Administrative	3.50	4.70	6.60	8.20	11.80
Interest (6% of fixed capital)	16.10	24.40	37.00	47.20	71.70
10% Return on investment	28.00	42.20	64.00	81.70	124.00
Hourly production cost	196.57	328.14	565.08	786.90	1410.40
lb product per hour	1540	3080	6160	9240	18480
Product production cost	12.90	10.65	9.20	8.55	7.64

Discussion of Results

The literature contains many examples of partial or complete acid hydrolysis of cellulose followed by microbial consumption of the hydrolysate. Very few examples of partial alkaline hydrolysis followed by microbial growth on the hydrolysate have been reported. Partial hydrolysis with acid usually results in a product more crystalline than the starting material but with a lower average degree of polymerization. Alkaline hydrolysis has almost the reverse effect. The product after partial hydrolysis is considerably less crystalline than the starting material and will have undergone no decrease in molecular weight unless oxygen was available during the treatment.

When growing a mixed culture of cellulomonas and Alcaligenes on a chemically treated bagasse the degree of crystallinity of the insoluble portion will effect only the growth of the cellulomonas since Alcaligenes does not consume molecules that are of sufficient degree of polymerization to enter into network structures. The total degree of polymerization of the feed will determine the distribution of the organisms in the product.

If the alkaline pretreatment is carried out in the complete absence of air or oxygen, the only effect the treatment will have had is to make those molecules that were formerly unavailable because of lignin enclosure or crystalline occlusion available to both organisms. Under these conditions, the growth rates of the two organisms will be inversely proportional to the degree of crystallinity. The growth rates will represent a maximum for the consumption of insoluble cellulose because the cellulase enzymes will be induced. On the other hand, if the oxidation is too

severe the cellulase enzymes will not be induced because of the presence of large quantities of small molecules that can diffuse through the walls of the cellulomonas and inhibit enzyme production.

If the product of hydrolysis is composed completely of soluble short chain polysaccharides, alcaligenes will grow very nicely. But it has been shown by many people that if the hydrolysis step has to be so severe that it causes complete solubilization negating therefore the requirement of enzymatic hydrolysis then the hydrolysis step will generally be so costly that the process is not economically feasible.

The investigators in this research had once thought that a two-stage fermentation may offer the best solution to the problem. The thinking was that the soluble carbohydrates which resulted from the alkali treatment could be consumed by alcaligenes in the first fermenter and since the overflow from it would have essentially no soluble carbohydrates, the second fermenter could have cellulomonas with its induced cellulases with no fear of inhibition from small molecules. It has since been concluded that two fermenters in series would be so subject to cycling that they may be impractical.

The new thinking is to form a minimum amount of soluble carbohydrates during alkali treatment with the maximum disruption of crystallinity and if necessary wash the cellulose after alkali treatment to remove as much of the soluble carbohydrates as necessary. If for some reason the feed stream contained large amounts of both soluble and insoluble carbohydrates then a 2 stage fermentation may possibly be the only answer but a hold-up vessel for storing up feed to the second fermenter would then be necessary.

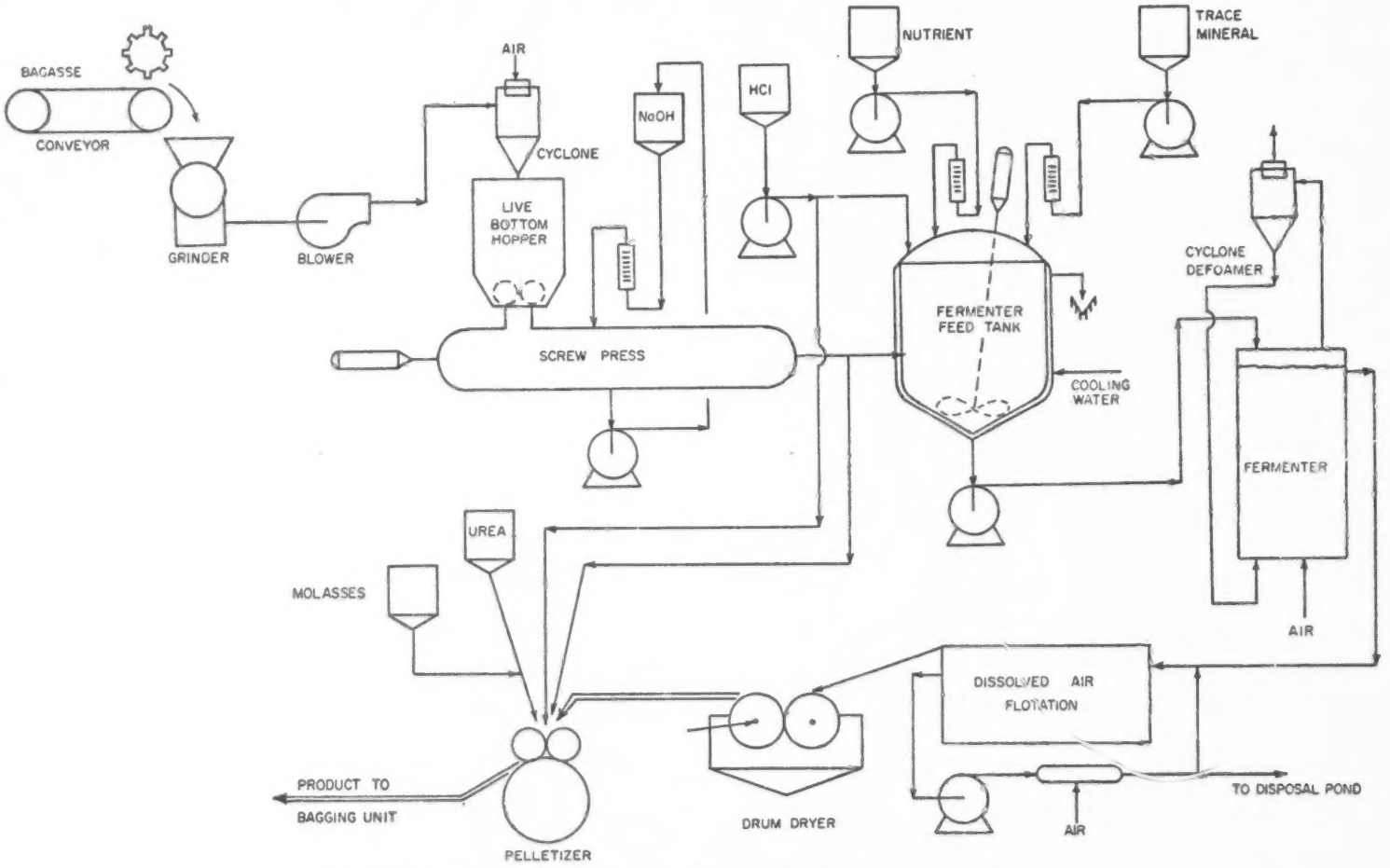
The process as it is now envisioned is complicated and requires a large capital investment. This in turn causes the product cost to be high

to pay the depreciation and interest costs. It is built almost entirely of stainless steel because this is an FDA requirement in food preparation. Little emphasis is currently being given to using this product for anything except animal feed. It may be wise to reconsider the process equipment in terms of feed rather than food product. If this were done several changes could be made that would reduce the equipment cost and therefore the product cost. Several pieces of equipment could be changed from stainless to carbon steel, this would cut the cost of pumps, tanks, fermenter, etc. almost in half and would cause a corresponding decrease in capital costs.

The plant may also be run under non-sterile conditions. This would cut the cost of the fermenters essentially in half, would eliminate the feed sterilization section and the air filters. If the product could contain cellulose, this would eliminate the product filter and cellulose recycle system, both expensive items.

Another important concept in this research is the fact that alkali treatment of the cellulose makes it more digestible to all microorganisms. This includes the microorganisms in a cow's stomach as well as those in a fermenter. In other words, alkali treatment can greatly enhance the digestibility of cellulosics to ruminants. This was reported by Stone (52) as well as others. A plant could be designed to have a large alkali treating capacity compared to the fermentation capacity (53). Most of the alkali treated cellulosic could by-pass the fermenters and other expensive equipment to be recombined with 10 to 15% of microbial cells at the end of the process as shown in Figure 12.

Urea and molasses could be added to the protein and alkali



PROPOSED PROCESSING PLANT FOR NUTRITIONALLY-BALANCED FEED

Figure 12

treated cellulose and all pelletized together to make a balanced ruminant feed. This technique of by-passing the fermenters, filter, floatation units, etc. will greatly decrease the cost of the final product.

To evaluate the potential that this approach might have, a new raw materials list and equipment list were made up for the proposed process and tabulated in Table XIII. From this data a manufacturing cost estimate was made as shown in Table XIV. This data shows that for a plant to produce 3600 tons per month of a balanced ruminant feed, the cost of production will be $50.00 per ton. This cost is very competitive with the cost of alfalfa and certainly would be as good a feed.

This entire project was started on the basis of some earlier work by several members of this research team and reported by E.J. Stone (52). This work showed that alkali pretreatment of bagasse increased the digestiblity of cellulose from 15% to 57% to cows (see Table II). Since a cow's stomachs are just microbial fermentation chambers, it was reasoned that alkali treated bagasse could serve as the substrate for growing microorganisms in fermenters to produce protein directly. It may be possible that a combination of these two approaches would offer the best solution to the problems of cellulose waste disposal and the world wide protein deficiency.

Table XIII

Additional Raw Materials and Equipment Needed to Produce a Balanced Ration for Ruminants

10,000 lb per hour - 3,600 tons per month

Raw Material	lb/hr	cents/lb	$ cost per hour
Bagasse	8000	0.25	20.00
NaOH	364	4.65	16.90
Hcl	330	1.30	4.30
Urea	200	3.2	6.40
Protein (15%)	1540	2.93	45.07
unreacted cellulose	300	0	0
		Total	92.67

Additional Equipment Cost		Increased Cost
Mg-1	magnetic separator	8,600
C-1	bagasse conveyor	8,500
G-1	bagasse grinders	15,000
H-1	live bottom hopper	12,000
T-5	alkali treating tank	30,000
M-1	motor for alkali tank	7,500
S-1	alkali squeeze press & drive	42,000
P-1	product pelletizer (granulator)	70,600
		194,800

Table XIV

Manufacturing Cost Estimate for 3,600 tons per month of Balanced Ruminant Ration
(Contains 15% protein supplement plus 2% urea)

Direct Production Cost	cost dollars/hr.
Raw Material	92.67
Utilities	74.00
Fixed Charges	
Depreciation	31.00
Insurance	3.42
Local Taxes	6.84
Plant Overhead	
Medical (etc)	15.50
General Expenses	
Administrative	4.90
Interest	20.50
Total	248.83

product 10,000 #/hr

product cost 2.49 cents per pound

or

$50.00 per ton

Conclusions

1. The economics of the process reported here have been calculated as carefully as possible on the limited data available. If the product becomes a commodity with reasonably large sales, the cost of production may decrease to less than that quoted.

2. Very little research has been done on any of this process other than alkali treatment and fermentation since it has been felt that if these steps were not practical there was no real need to work on the others. More research on the remaining steps would now seem to be justified.

3. The most costly single step in the process appears to be that of drying the cells. This cost of 1.2 cents per pound of product may possibly be decreased by research in the area of cell flocculation, flotation or hyperfiltration.

4. The economics of the process are strongly dependent on how completely the caustic is removed after the alkali treatment step. Research is also warrented in this area.

5. One ojbective of the alkali pretreatment is to depolymerize the lignin encapsulating the cellulose, a second is to decrystallize the cellulose. If these objectives are accomplished with a minimum decrease in the molecular weight of the cellulose, good growth rates will result.

6. A large decrease in the degree of crystallinity can be obtained by the use of any or all of the following:

a. high temperature

b. high caustic concentration

c. long times at intermediate temperatures and caustic concentrations

d. oxidation catalysts such as cobaltous chloride

e. oxidants such as hydrogen peroxide, NaOCl, etc.

7. Complete acid hydrolysis of cellulose to a soluble substrate for conversion to yeast protein may not be practical when compared to the production of single cell protein using a cellulolytic organism.

8. Partial acid hydrolysis of the cellulose followed by microbial consumption may also not be practical because acid hydrolysis occurs in the amorphous regions leaving behind the crystalline areas. Highly crystalling cellulose can only be attacked at the surface of the crystallites by cellulolytic enzymes and the amount of this surface is so limited that microbial attack is very slow.

9. Dilute alkali acts as a partial solvent for cellulose causing a decrease in the degree of crystallinity with treatment rather than an increase as occurs with acid treatment. Therefore a two step process involving alkali pretreatment,but not necessarily hydrolysis, may probably represent the most practical approach.

10. Competitive methods of disposal of waste cellulose such as mixing it with activated sludge and going through an anaerobic digestion may be too slow to be practical and, furthermore, the product has no real value.

11. The enzymatic digestion of waste cellulose using the cellulolytic enzymes from trichoderma viride QM-6A as suggested by Golueke (15) may also not be economically practical because a long anaerobic digestion period of about 8 days is required and this is followed by an aerobic digestion to grow yeast. Meller has shown that a two stage process for yeast production involving a complete prehydrolysis of the cellulose may be too expensive to be practical.

Recommendations

1. The reason the present process gives as high a production cost as it does is primarily due to the cost of processing equipment rather than to low volumetric production efficiency. Since alkali processing appears to be a practical method of recycle of cellulosic wastes, effort should be started to simplify and improve the process.

2. A high percentage of protein in the product is probably of limited value for animal feeds. The process for cell harvest could be greatly simplified if the unreacted cellulose could be combined with the protein product. This would cut processing costs greatly and may even improve the product if fed to ruminants.

3. It should not be forgotten that nature has made cow's stomachs into microbial chambers where cellulose is enzymatically hydrolyzed to soluble sugars. The lessons learned in this research on how to treat cellulose to make it most susceptible to enzymatic digestion should be used to produce feed for ruminants.

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